DESCRIPTION

POLYPEPTIDES, THEIR PRODUCTION AND USE

[Technical Field]

The present invention relates to a novel ligand polypeptide for the G protein-coupled receptor protein and a DNA comprising a DNA encoding the ligand polypeptide.

[Background Art]

Many hormones and neurotransmitters mediate biological functions through specific receptors present on the cell membrane. Many of these receptors engage themselves in the intracellular transduction of signals through activation of the coupled guanine nucleotide-binding protein (hereinafter sometimes referred to briefly as G protein) and have the common structure comprising 7 transmembrane domains. Therefore, these receptors are collectively referred to as G protein-coupled receptor or 7-transmembrane receptor.

One of the pathways to modulate biological functions mediated by such hormones or neurotransmitters through G protein-coupled receptors is the hypothalamo-pituitary system. Thus, the secretion of pituitary hormone from the hypophysis is controlled by hypothalamic hormones (pituitatropic releasing factor) and the functions of the target cells or organs are regulated through the pituitary hormones released into the circulation. This pathway carries out functional modulations of importance to the living body, such as homeostasis and regulation of the reproduction, development, metabolism and growth of individuals. The secretion of pituitary hormones is controlled by a positive feedback or a negative feedback mechanism involving hypothalamic hormone and the peripheral hormone secreted from the target endocrine gland. The

various receptor proteins present in the hypophysis are playing a central role in the regulation of the hypothalamus-pituitary system.

Meanwhile, it is known that these hormones and factors as well as their receptors are not localized in the hypothalamus-pituitary system but are broadly distributed in the brain. Therefore, it is suspected that, in the central nervous system, this substance called hypothalamus hormone is functioning as a neurotransmitter or a neuromodulator. Moreover, the substance is distributed in peripheral tissues as well and thought to be playing important roles in the respective tissue.

The pancreas is playing a crucial role in the carbohydrate metabolism by secreting glucagon and insulin as well as digestive juice. While insulin is secreted from the pancreatic β cells, its secretion is mainly stimulated by glucose. However, it is known that β cells have a variety of receptors and the secretion of insulin is controlled by a number of factors in addition to glucose as well as peptide hormones, e.g. galanine, somatostatin, gastric inhibitory polypeptide, glucagon, amyrin, etc.; sugars, e.g. mannose etc.; amino acids, and neurotransmitters, among others.

The means only heretofore available for identifying ligands for said G protein-coupled receptor proteins is estimation from the homology in primary structure of G protein-coupled receptor proteins.

Recently, investigation for novel opioid peptides by introducing a cDNA coding for a receptor protein which a ligand is unknown, i.e. an orphan G protein-coupled receptor protein, into animal cells have been reported (Reinsheid, R. K. et al., Science, 270, 792-794, 1995, Menular, J.-C., et al., Nature 377, 532-535, 1995). However, in view of similarities to known G

protein-coupled receptor proteins and tissue distributions, it could be easily anticipated in these cases that the ligand would be belonging to the family of opioid peptides. The history of research and development in the realm of substances acting on the living body through the opioid receptor dates back to many years ago and various antagonists and agonists had been developed. Therefore, among the compounds artificially synthesized, an agonist of the receptor was picked out and, using it as a probe, expression of the receptor in the receptor cDNA-transfected cells was verified. Then, a search was made for an activator of the intracellular signal transduction which was similar to the agonist, the activator so found was purified, and the structure of the ligand was determined. However, when the homology of an orphan receptor to known G protein-coupled receptor proteins is low, it was very difficult to predict its ligand.

Ligands for orphan G protein-coupled receptors expressed in the hypophysis, central nervous system, and pancreatic β cells are considered to be useful for developing medicines, but their structures and functions have not been elucidated as yet.

[Disclosure of Invension]

Employing a cell in which a cDNA coding for orphan G protein-coupled receptor protein has been expressed by a suitable means and using measurement of a specific cell stimulation activity exemplified by a signal transduction activity as an indicator, the inventors of the present invention succeeded in screening a polypeptide which said receptor protein recognizes as a ligand.

Furthermore, the inventors found that a compound can be screened which is capable of changing the binding activity of this ligand which is an activating

factor to said receptor protein.

The present invention, therefore, relates to

(1) A polypeptide which comprises an amino acid sequence represented by SEQ ID NO:73 or its substantial equivalent thereto, or its amide or ester, or a salt thereof.

(2) The polypeptide as described in (1) above, which comprises the amino acid sequence represented by SEQ ID NO:3, SEC ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, or SEQ ID NO:66.

(3) The polypeptide as described in (1) above, which comprises the amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:44, SEQ ID NO:45, or SEQ ID NO:59.

(4) A partial peptide of the polypeptide as described in (1) above its amide or ester, or a salt thereof.

(5) A DNA which comprises a DNA having a nucleotide sequence coding for the polypeptide as described in (1) above or the partial peptide as described in (4) above.

(6) The DNA as described in (5) above which comprises a nucleotide sequence represented by SEQ ID NO:2, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:18, SEQ ID NO:46, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, or SEQ ID NO:72.

(7) A recombinant vector comprising the DNA as described in (5) above.

(8) A transformant carrying the DNA as described in (5) above or the recombinant vector as described (7) above.

(9) A method for producing the polypeptide as described in (1) above or the partial peptide as

described in (4) above, which comprises culturing the transformant as described in (8) above.

(10) A pharmaceutical composition containing the polypeptide, its amide or ester as described in (1) above, or a pharmaceutically acceptable salt thereof.

(11) A pharmaceutical composition containing the partial peptide peptide, its amide or ester as described in (4) above, or a pharmaceutically acceptable salt thereof.

(12) A pharmaceutical composition containing the DNA as described in (5) above.

(13) The pharmaceutical composition as described in (10), (11), or (12) above, which is a pituitary function modulator.

(14) The pharmaceutical composition as described in (10), (11), or (12) above, which is a central nervous system function modulator.

(15) The pharmaceutical composition as described in (10), (11), or (12) above, which is a pancreatic function modulator.

(16) An antibody against the polypeptide as described in (1) above or against the partial peptide as described in (4) above.

(17) A screening method for a compound capable of changing the binding activity of the polypeptide as described in (1) above or the partial peptide as described in (4) above, with a receptor protein comprising an amino acid sequence represented by SEQ ID NO:21 or its partial peptide or its substantial equivalent thereto, or a salt thereof, which comprises making a comparison between: (i) at lease one case where said polypeptide as described in (1) above or the partial peptide as described in (4) above is contacted with a receptor protein comprising an amino acid sequence represented by SEQ ID:21 or its partial peptide or its substantial equivalent thereto, or a

salt thereof, and (ii) at least one case where said polypeptide as described in (1) above or the partial peptide as described in (4) above together with a sample to be tested in contacted with protein comprising an amino acid sequence represented by SEQ ID NO:21 or its partial peptide or its substantial equivalent thereto, or a salt thereof.

(18) A kit for screening for a compound capable of changing the binding activity of the polypeptide as described in (1) above or the partial peptide as described in (4) above with a receptor protein comprising an amino acid sequence represented by SEQ ID NO:21 or its partial peptide or its substantial equivalent thereto, or a salt thereof.

(19) A compound capable of changing the binding activity of the polypeptide as described in (1) or the partial peptide as described in (4) with a receptor protein comprising an amino acid sequence represented by SEQ ID NO:21 or its partial peptide or its substantial equivalent thereto, or a salt thereof.

(20) A G protein-coupled receptor protein which recognizes the polypeptide as described in (1) above or the partial peptide as described in (4) above as a ligand, or a salt thereof.

The present invention further provides:

(21) the polypeptide as described in (1) above, or its amide or ester, or a salt thereof, which comprises an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:73, amino acid sequences wherein 1 to 15 amino acid residues, preferably 1 to 10 amino acid residues, more preferably 1 to 5 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:73, amino acid sequences wherein 1 to 80 amino acid residues, preferably 1 to 50 amino acid residues, more preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID

NO:73, and amino acid sequences wherein 1 to 15 amino acid residues, preferably 1 to 10 amino acid residues, more preferably 1 to 5 amino acid resides in the amino acid sequence of SEQ ID NO:73 are substituted with one or more other amino acid residues;

(22) the polypeptide as described in (1) above, which comprises an amino acid sequence wherein the peptide of SEQ ID NO:74 is added to the N-terminus of the polypeptide comprising the amino acid sequence of SEQ ID NO:73;

(23) the polypeptide as described in (1) above, which in derived from bovine, rat or human; and

(24) the pharmaceutical composition described in (10), (11) or (12) above, which is a therapeutic and/or prophylactic agent for dementia, depression (melancholia), hyperkinetic (microencephalo-pathy) syndrome, disturbance of consciousness, anxiety syndrome, schizophrenia, horror, growth hormone secretory disease, hyperphagia, polyphagia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, hyperprolactinemia, diabetes, cancer, pancreatitis, renal disease, Turner's syndrome, neurosis, rheumatoid arthritis, spinal injury, transient brain ischemia, amyotrophic lateral sclerosis, acute myocardial infarction, spinocerebellar degeneration, bone fracture, trauma, atopic dermatitis, osteoporosis, asthma, epilepsy, infertility and/or oligogalactia.

Referring to the G protein-coupled receptor protein for the ligand polypeptide in accordance with the present invention, the invention specifically provides:

(25) the G protein-coupled receptor protein described in (20) or a salt thereof, which comprises an amino acid sequence represented by SEQ ID NO:19 or its substantial equivalent thereto or/and an amino acid sequence represented by SEQ ID NO:20 or its substantial

equivalene thereto;

(26) the G protein-coupled receptor protein described in (25) above or a salt thereof, which comprises an amino acid sequence represented by SEQ ID NO:21 or its substantial equivalent thereto;

(27) the G protein-coupled receptor protein described in (25) above or a salt thereof, which comprises an amino acid sequence represented by SEQ ID NO:22 or its substantial equivalent thereto;

(28) the G protein-coupled receptor protein described in (25) above or a salt thereof, which comprises an amino acid sequence represented by SEQ ID NO:23 or its substantial equivalent thereto;

(29) a partial peptide of any of the G protein-coupled receptor proteins described in (25)-(28) above or a salt thereof;

(30) a DNA which comprises a DNA having a nucleotide sequence coding for the G protein-coupled receptor protein described in (25) above;

(31) a DNA which comprises a DNA having a nucleotide sequence coding for the G protein-coupled receptor protein described in (26) above;

(32) a DNA which comprises a DNA having a nucleotide sequence coding for the G protein-coupled receptor protein described in (27) above;

(33) a DNA which comprises a DNA having a nucleotide sequence coding for the G protein-coupled receptor protein described in (28) above;

(34) the DNA described in (30) above, which comprises the nucleotide sequence of SEQ ID NO:24 or the nucleotide sequence of SEQ ID NO:25;

(35) the DNA described in (31) above, which comprises the nucleotide sequence of SEQ ID NO:26;

(36) the DNA described in (32) above, which comprises the nucleotide sequence of SEQ ID NO:27;

(37) the DNA described in (33) above, which comprises

the nucleotide sequence of SEQ ID NO:28;
(38) a recombinant vector comprising any of the DNAs described in (30)-(33) above;
(39) a transformant carrying the recombinant vector described in (38) above;
(40) a method for producing the G protein-coupled receptor protein described in (28) above or a salt thereof, which comprises culturing the transformant of (39) to produce said G protein-coupled receptor protein on the cell membrane of the transformant;
(41) an antibody to any of the G protein-coupled receptor protein described in (25)-(28) above or a salt thereof, or the partial peptide described in (29) above or a salt thereof.

To be further specific, the G protein-coupled receptor protein relates to:
(42) the G protein-coupled receptor protein described in (25) above or a salt thereof, wherein the protein comprises (i) an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:19, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:19, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:19, and amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:19 are substituted with one or more than amino acid residues and/or (ii) an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:20, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:20, amino acid sequences wherein 1 to 30 amino acid

residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:20, and amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:20 are substituted with one or more other amino acid residues;

(43) the G protein-coupled receptor protein described in (26) above or a salt thereof, wherein the protein comprises an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:21, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:21, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:21, and amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:21 are substittued with one or more other amino acid residues;

(44) the G protein-coupled receptor protein described in (27) above or a salt thereof wherein the protein comprises an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:22, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:22, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:22, and amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:22 are substituted with one or more other amino acid residues;

(45) the G protein-coupled receptor protein described in (28) above or a salt thereof, wherein the protein

comprises an amino acid sequence selected from the group consisting of an amino acid sequence of SEQ ID NO:23, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:23, amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:23, and amino acid sequences wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:23 are substituted with one or more other amino acid residues.

As used herein the term "substantial equivalent(s)" means that the activity of the protein, e.g., nature of the binding activity of the ligand and the receptor and physical characteristics are substantially the same. Substitutions, deletions or insertions of amino acids often do not produce radical changes in the physical and chemical characteristics of a polypeptide, in which case polypeptides containing the substitution, deletion, or insertion would be considered to be substantially equivalent to polypeptides lacking the substitution, deletion, or insertion. Substantially equivalent substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

[Brief Description of the Drawings]

Fig. 1 shows the nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA and the amino acid encoded by the nucleotide sequence. The primer used for sequencing was -21M13. The underscored region correspond to the synthetic primer.

Fig. 2 shows the nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in cDNA clone p19P2 isolated by PCR using human pituitary-derived cDNA and the amino acid sequence encoded thereby. The primer used for sequencing was M13RV-N (Takara). The underscored region correspond to the synthetic primer.

Fig. 3 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in Fig. 1.

Fig. 4 shows a partial hydrophobic plot of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 constructed according to the amino acid sequence shown in Fig. 2.

Fig. 5 is a diagram comparing the partial amino acid sequence of the protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment harbored in p19P2 as shown in Figs. 1 and 2 with the known G protein-coupled receptor protein S12863. The shadowed region represents the region of agreement. The 1 to 9 amino acid sequence of p19P2 corresponds to the 1 to 99 amino acid sequence of Fig. 1 and the 156 to 230 amino acid sequence corresponds to the 1 to 68 amino acid sequence of Fig. 2.

Fig. 6 shows the nucleotide sequence of the MIN6-

derived G protein-coupled receptor protein cDNA fragment based on the nucleotide sequences of the MIN6-derived G protein-coupled receptor protein cDNA fragments harbored in the cDNA clones pG3-2 and pG1-10 isolated by PCR using MIN6-derived cDNA and the amino acid sequence encoded by the nucleotide sequence. The underscored region correspond to the synthetic primer.

Fig. 7 is a diagram comparing the partial amino acid sequence encoded by pG3-2/pG1-10 of the MIN6-derived G protein-coupled receptor protein shown in Fig. 6 with the partial amino acid sequence of the protein encoded by p19P2 shown in Figs. 1 and 2. The shadowed region corresponds to the region of agreement. The 1 to 99 amino acid sequence of the protein encoded by p19P2 corresponds to the 1 to 99 amino acid sequence of Fig. 1 and the 156 to 223 amino acid sequence corresponds to the 1 to 68 amino acid sequence of Fig. 2. The 1 to 223 amino acid sequence of the protein encoded by pG3-2/pG1-10 corresponds to the 1 to 223 amino acid sequence of Fig. 6.

Fig. 8 is a partial hydrophobic plot of the MIN6-derived G protein-coupled receptor protein constructed according to the partial amino acid sequence shown in Fig. 6.

Fig. 9 shows the entire nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA harbored in the cDNA clone phGR3 isolated from a human pituitary-derived cDNA library by the plaque hybridization method using the DNA fragment inserted in p19P2 as a probe and the amino acid sequence encorded by the nucleotide sequence.

Fig. 10 shows the result of Northern blotting of human pituitary mRNA hybridized with radioisotope-labeled human pituitary cDNA clone phGR3.

Fig. 11 shows a hydrophobic plot of the protein encoded by the human pituitary-derived G protein-

coupled receptor protein cDNA harbored in the phGR3 as constructed according to the amino acid sequence shown in Fig. 9.

Fig. 12 shows the nucleotide sequence of the MIN6-derived G protein-coupled receptor protein cDNA fragment harbored in the cDNA clone p5S38 isolated by PCR using MIN6-derived cDNA and the amino acid sequence encoded by the nucleotide sequence. The underscored region correspond to the synthetic primer.

Fig. 13 shows a diagram comparing the partial amino acid sequence of MIN6-derived G protein-coupled receptor protein encoded by p5S38 shown in Fig. 12 with the partial amino acid sequence of G protein-coupled receptor protein encoded by the cDNA fragment harbored in p19P2 as shown in Figs. 1 and 2 and the partial amino acid sequence of G protein-coupled receptor protein encoded by the nucleotide sequence generated from the nucleotide sequences of cDNA fragments contained in pG3-2 and pG1-10 shown in Fig. 6. The shadowed region represents the sequence region of agreement. The 1 to 144 amino acid sequence of the protein encoded by p5S38 corresponds to the 1 to 144 amino acid sequence of Fig. 12, the 1 to 99 amino acid sequence of the protein encoded by p19P2 corresponds to the 1 to 99 amino acid sequence of Fig. 1 and the 156 to 223 amino acid sequence corresponds to 1 to 68 amino acid sequence of Fig. 2. The 1 to 223 amino acid sequence of the protein encoded by pG3-2/pG1-10 corresponds to the 1 to 223 amino acid sequence of Fig. 6.

Fig. 14 shows a partial hydrophobic plot of the protein encoded by the MIN6-derived G protein-coupled receptor protein cDNA harbored in p5S38 as constructed according to the partial amino acid sequence shown in Fig. 12.

Fig. 15 shows the results of the following

analysis. Thus, RT-PCR was carried out to confirm the expression of mRNA in CHO cells transfected by pAKKO-19P2. Lanes 1-7 represent the results obtained by performing PCRs using serial dilutions of pAKKO-19P2 for comparison, i.e. the 10μl/ml stock solution (lane 1), 1/2 dilution (lane 2), 1/4 dilution (lane 3), 1/64 dilution (Lane 4), 1/256 dilution (lane 5), 1/1024 dilution (lane 6), and 1/4096 dilution (lane 7) of the plasmid as templates, and analyzing the reaction mixtures by 1.2% agarose gel electrophoresis. Lanes 8 through 11 are the results obtained by performing PCRs using a 1/10 dilution (lane 8), a 1/100 dilution (lane 9), and a 1/1000 dilution (lane 10) of the cDNA prepared from the CHO-19P2 cell line as templates and subjecting the respective reaction mixtures to electrophoresis. Lane 11 was obtained by performing PCR using a template obtained by carrying out cDNA synthesis without reverse transcriptase and subjecting the PCR reaction product to electrophoresis. Lanes 12 and 13 were obtained by performing PCR using cDNAs prepared from mock CHO cells with and without addition of reverse transcriptase, respectively, as templates and subjecting the respective reaction products to electrophoresis. M represents the DNA size marker. The lanes at both ends were obtained by electrophoresing 1 μl of λ/Sty I digest (Nippon Gene) and the second lane from right was obtained with 1 μl of ø/χ174/Hinc II digest (Nippon Gene). The arrowmark indicates the position of the band amplified by PCR of about 400 bp.

Fig. 16 shows the activity of the crude ligand peptide fraction extracted from rat whole brain to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite releasing activity was expressed as % of the amount of [^{3}H] arachidonic acid metabolites released in the

presence of the crude ligand polypeptide fraction with the amount of [^{3}H] arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in a 30% CH_3CN fraction.

Fig. 17 shows the activity of the crude ligand polypeptide fraction extracted from bovine hypothalamus to promote release of arachidonic acid metabolites from CHO-19P2 cells. The arachidonic acid metabolite release-promoting activity was expressed as % of the amount of [^{3}H] arachidonic acid metabolites released in the presence of the crude ligand polypeptide fraction with the amount of [^{3}H] arachidonic acid metabolites released in the presence of 0.05% BAS-HABB being taken as 100%. The activity to promote release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in a 30% CH_3CN fraction just as in the crude ligand polypeptide fraction from rat whole brain.

Fig. 18 shows the activity of the fraction purified with the reversed-phase column C18 218TP5415 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The active fraction from RESOURCE S was fractionated on C18 218TP5415. Thus, chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 20%-30% CH_3CN/0.1% TFA/H_2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity of each fraction to specifically promote release of arachidonic acid metabolites from the CHO-19P2 cell line was determined. As a result, the activity was fractionated into 3 fractions (designated, in the order of elution, as P-1, P-2, and P-3).

Fig. 19 shows the activity of the fraction purified with the reversed-phase column diphenyl

219TP5415 to specifically promote arachidonic acid metabolite release from CHO-19P2 cells. The P-3 active fraction from C18 218TP5415 was fractionated on diphenyl 219TP5415. The chromatography was carried out at a flow rate of 1 ml/min. on a concentration gradient of 22%-25% CH_3CN/0.1% TFA/H_2O, the eluate was collected in 1 ml fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity converged in a single peak.

Fig. 20 shows the activity of the fraction purified by reversed-phase column μRPC C2/C18 SC 2.1/10 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The peak active fraction from diphenyl 219TP5415 was fractionated on μRPC C2/C18 SC 2.1/10. The chromatography was carried out at a flow rate of 100 μl/min. on a concentration gradient of 22%-23.5% CH_3CN/0.1% TFA/H_2O, the eluate was collected in 100 μl fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity was found as two peaks of apparently a single substance (peptide).

Fig. 21 shows the activity of the P-2 fraction purified by reversed-phase column μRPC C2/C18 SC 2.1/10 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The chromatography was carried out at a flow rate of 100 μl/min. on a concentration gradient of 21.5%-23.0% CH_3CN/0.1% TFA/dH_2O, the eluate was collected in 100 μl fractions, and each fraction was lyophilized. Then, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in each fraction was determined. As a result, the activity was found as a

peak of apparently a single substance.

Fig. 22 shows the nucleotide sequence of bovine hypothalamus ligand polypeptide cDNA fragment as derived from the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

Fig. 23 shows the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment generated according to the nucleotide sequence of the bovine hypothalamus-derived ligand polypeptide cDNA fragment which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as harbored in a cDNA clone isolated by PCR using bovine hypothalamus-derived cDNA and the amino acid sequence encoded by said nucleotide sequence. The region indicated by the arrowmark corresponds to the synthetic primer.

Fig. 24 shows the amino acid sequences (a) and (b) of the bovine hypothalamus-derived ligand polypeptides which specifically promote release of arachidonic acid metabolites from CHO-19P2 cells and the cDNA sequence coding for the full coding region of the ligand polypeptides defined by SEQ ID NO:1 and SEQ ID NO:44.

Fig. 25 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic peptide was dissolved in degassed dH_2O at a final concentration of 10^{-3}M and diluted with 0.05% BSA-HBSS to concentrations of 10^{-12}M-10^{-6}M. The arachidonic acid metabolite releasing activity was expressed in the

measured radioactivity of [^{3}H] arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-31 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a concentration-dependent manner.

Fig. 26 shows the concentration-dependent activity of synthetic ligand polypeptide (19P2-L31(O)) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic ligand peptide was dissolved in degassed dH_2O at a final concentration of 10^{-3}M and diluted with 0.05% BSA-HBSS to concentrations of 10^{-12}M-10^{-6}M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of [^{3}H] arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L31(O) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a dose-dependent manner.

Fig. 27 shows the activity of synthetic ligand polypeptide 19P2-L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells. The synthetic peptide was dissolved in degassed dH_2O at a final concentration of 10^{-3}M and diluted with 0.05% BSA-HBSS to concentrations of 10^{-12}M-10^{-6}M. The arachidonic acid metabolite releasing activity was expressed in the measured radioactivity of [^{3}H] arachidonic acid metabolites released in the supernatant when the dilution was added to the cells. As a result, the activity of 19P2-L20 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was found in a dose-dependent manner.

Fig. 28 shows the 1.2% agarose gel electrophoregram of the DNA fragments of the phages

cloned from a bovine genomic library as digested with restriction enzymes BamHI(B) and SalI(S). As the DNA size marker (M), StyI digests of λ phage DNA were used. In lane B, two bands derived from the vector were detected in positions between the first (19,329 bp) and second (7.743 bp) marker bands, as well as 3 bands derived from the inserted fragment between the third (6,223 bp) and 5th (3,472 bp) bands. In lane S, two bands derived from the vector were similarly detected but due to the overlap of the band of the inserted fragment, the upper band is thicker than the band in lane B.

Fig. 29 shows the nucleotide sequence around the coding region as decoded from bovine genomic DNA. The 1st to 3rd bases (ATG) correspond to the translation start codon and the 767th to 769th bases (TAA) correspond to the translation end codon.

Fig. 30 shows a comparison between the nucleotide sequence (genome) around the coding region as deduced from bovine genomic DNA and the nucleotide sequence (cDNA) of bovine cDNA cloned by PCR. The sequence region of agreement is indicated by shading. As to the 101st to 572nd region, there is no corresponding region in the nucleotide sequence of cDNA, indicating that it is an intron.

Fig. 31 shows the translation of the amino acid sequence encoded after elimination of the intron from the nucleotide sequence around the coding region as decoded from bovine genomic DNA.

Fig. 32 shows the full-length amino acid sequence and the cDNA sequence coding for the full coding region of rat ligand polypeptide.

Fig. 33 shows amino acid sequence of bovine ligand polypeptide and the nucleotide sequences of DNAs coding for bovine polypeptide and rat polypeptide. The arrowmark indicates the region corresponding to the

synthetic primer.

Fig. 34 shows the full-length amino acid sequence and the sequence of cDNA coding for the full coding region of human ligand polypeptide.

Fig. 35 shows a comparison of the amino acid sequences in the translation region of bovine ligand polypeptide, rat ligand polypeptide, and human ligand polypeptide.

Fig. 36 shows the results of receptor binding experiments on living cells wherein radioiodinated ligand polypeptide is used in the experiments.

Fig. 37 shows the results of measurements of release of arachidonic acid metabolites from CHO-19P2-9 and CHO-UHR1 by ligand polypeptide.

Fig. 38 shows the results of quantification of UHR-1 mRNA by RT-PCR in discrete regions of the brain and tissues in rats.

Fig. 39 shows the results of quantification of ligand polypeptide mRNA by RT-PCR in discrete regions of the brain and tissues in rats.

Fig. 40 shows effects of ligand polypeptide on glucose-induced increase in plasma insulin concentration, which is measured by radioimmunoassay.

Fig. 41 shows the results of measurements of motor activity by administration of 10 nmol of ligand polypeptide to mouse.
(a) relates to spontaneous motor activity and (b) relates to rearing.

Fig. 42 shows the results of measurements of motor activity by administration of 1 nmol of ligand polypeptide to mouse.
(a) relates to spontaneous motor activity and (b) relates to rearing.

Fig. 43 shows the results of measurements of motor activity by administration of 0.1 nmol of ligand polypeptide to mouse.

(a) relates to spontaneous motor activity and (b) relates to rearing.

Fig. 44 shows the results of measurements of motor activity by administration of 0.01 nmol of ligand polypeptide to mouse.

(a) relates to spontaneous motor activity and (b) relates to rearing.

Fig. 45 shows the results of measurements of body temperature which is measured at the time when the ligand polypeptide is administered to the lateral ventricle of mice. The administration of ligand polypeptide is carried out after 15 hours from administration of reserpine at a dose of 3 mg/kg, S.C.

In Fig. 45, the single star mark asterisk shows $p<0.05$ and the double star marks asterisks shows $p<0.01$.

Fig. 46 illustrates the drawing in which the micro-injection cannula is inserted into the area postrema at an angle of 20°.

Fig. 47 shows the typical example of direct and average blood pressure which is measured after the injection of ligand polypeptide into the area postrema of rat. It is measured after the injection of 10 nmol of ligand polypeptide at the rate of 1 µl/min, and under the condition of non-anesthesia.

Fig. 48 shows the results of measurements of growth hormone (GH) in plasma when 50 nmol of ligand polypeptide is administered into the third ventricle of rat after anesthesia by pentobarbital.

Fig. 49 shows the changes of secretion of GH in plasma by administration of 50 nmol of ligand polypeptide into the third ventricle in freely moving rats.

The ligand polypeptide or PBS was administered into the third ventricle. At 10 min later, 5 µg/kg of GHRH was administered intravenously to the rat

conscious. GH levels were measured just prior to intraventricular administration (time 0) and 10, 20, 30, 40, and 60 min after the intravenous injection of GHRH.

In Fig. 49, the single star mark asterisk shows $p<0.05$ and the double star marks asterisks show $p<0.01$.

Fig. 50 shows the relationship between the ligand polypeptide serum and the absorbance.

Fig. 51 shows the inhibition of the release of archidonic acid metabolites by anti-ligand polypeptide polyclonal antibody.

Fig. 52 shows the sequence of cDNA coding for UHR-1, which is constructed on pAKKO-UHR1-7.

[Best Mode for Carrying Out the Invention]

The ligand polypeptide according to the present invention is a polypeptide which is capable of binding to G protein-coupled receptor protein and comprising an amino acid sequence represented by SEQ ID NO:73 or its substantial equivalent thereto or a partial peptide thereof, or its amide or ester, or a salt thereof. In SEQ ID NO:73, Xaa at 10th position is Ala or Thr; Xaa at 11th position is Gly or Ser; and Xaa at 21th position is H, Gly, or GlyArg.

The above ligand polypeptide, its amide or ester, or a salt thereof (hereinafter sometimes referred to briefly as the ligand polypeptide or the polypeptide), processes for their production, and uses for the polypeptide are now described in detail.

The above ligand polypeptide of the present invention includes any polypeptides derived from any tissues, e.g. pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, gall bladder, bone marrow, adrenal gland, skin, muscle, lung, digestive canal, blood vessel, heart, etc.; or cells of man and other warm-blooded animals, e.g. guinea pig, rat, mouse,

swine, sheep, bovine, monkey, etc. and comprising an amino acid sequence represented by SEQ ID NO:73 or its substantial equivalent thereto. For example, in addition to the protein comprising the amino acid sequence of SEQ ID NO:73, the ligand polypeptide of the present invention includes the protein comprising an amino acid sequence having a homology of about 50-99.9%, preferably 70-99.9%, more preferably 80-99.9% and especially preferably 90-99.9% to the amino acid sequence of SEQ ID NO:73 and having qualitatively substantially equivalent activity to the protein comprising the amino acid sequence of SEQ ID NO:73. The term "substantially equivalent" means the nature of the receptor-binding activity, signal transduction activity and the like is equivalent. Thus, it is allowable that even differences among grades such as the strength of receptor binding activity and the molecular weight of the polypeptide are present.

To be more specific, the ligand polypeptide of the present invention includes the polypeptide derived from the rat whole brain, bovine hypothalamus, or human whole brain and comprising the amino acid sequence of SEQ ID NO:73. In addition, the ligand polypeptide of the present invention includes the polypeptides which comprises substantial equivalent polypeptides such as polypeptides wherein 1 to 15, preferably 1 to 10, and more preferably 1 to 5 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:73, polypeptides wherein 1 to 80, preferably 1 to 50, more preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:73, or polypeptides wherein 1 to 15, preferably 1 to 10, more preferably 1 to 5 amino acid residues are substituted with one or more other amino acid residues.

The amino acid sequence of SEQ ID NO:73 comprises SEQ ID NO:8, 9, 10, 50, 51, 52, 64, 65 or 66. The

substantial equivalent polypeptides to the polypeptide comprising the amino acid sequence of SEQ ID NO: 73 are polypeptides comprising the amino acid sequences of SEQ ID NO:1, 3, 4, 5, 6, 7, 44, 45, 47, 48, 49, 59, 61, 62, or 63.

Among them, preferred is the polypeptide comprising the amino acid sequence of SEQ ID NO:73 and the polypeptide comprising the amino acid sequence which a peptide of SEQ ID NO:74 is added to the N-terminus of the polypeptide comprising the amino acid sequence of SEQ ID NO:73.

Furthermore, the polypeptide or partial peptide of the present invention includes those wherein the N-terminal side of Gln is cleaved in vivo to form pyroglutamyl peptide.

The peptides described in this specification, the left ends are the N-terminus (amino terminus) and the right end is the C-terminus (carboxyl terminus) according to the convention of the peptide indication. While the C-terminus of the polypeptide of SEQ ID NO:73 is usually carboxyl (-COOH) or carboxylate ($-COO^-$), it may be amide ($-CONH_2$) or ester (-COOR) form. The ester residue R includes a C_{1-6} alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc., a C_{3-8} cycloalkyl group such as cyclopentyl, cyclohexyl, etc., a C_{6-12} aryl group such as phenyl, α-naphthyl, etc., and a C_{7-14} aralkyl group such as a phenyl-C_{1-2} alkyl group, e.g. benzyl, phenethyl, benzhydryl, etc. or an α-naphthyl-C_{1-2} alkyl, e.g. α-naphthylmethyl etc. In addition, the ester may be a pivaloyloxymethyl ester which is broadly used for oral administration. When the polypeptide of SEQ ID NO:73 has a carboxyl or carboxylate group in any position other than the C-terminus, the corresponding amide or ester are also included in the concept of the polypeptide of the present invention. The ester mentioned just above

includes the esters mentioned for the C-terminus.

The preferred ligand polypeptide of the present invention is a peptide which the C-terminus is amidated. Especially preferred is a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 8, 47, 50, 61 or 64 which the C-terminus is amidated.

The salt of polypeptide of the present invention includes salts with physiologically acceptable bases, e.g. alkali metals or acids such as organic or inorganic acids, and is preferably a physiologically acceptable acid addition salt. Examples of such salts are salts thereof with inorganic acids, e.g. hydrochloric acid, phosphoric acid, hydrobromic acid or sulfuric acid, etc. and salts thereof with organic acids, e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid or benzenesulfonic acid, etc.

The ligand polypeptide or amide or ester, or a salt thereof of the present invention may be manufactured from the tissues or cells of warm-blooded animals inclusive of human by purifying techniques or manufactured by the peptide synthesis as described hereinafter. Moreover, it can be manufactured by culturing a transformant carrying a DNA coding for the polypeptide as described hereinafter.

In the production from the tissues or cells of human or other warm-blooded animals, the ligand polypeptide can be purified and isolated by a process which comprises homogenizing the tissue or cells of human or other warm-blooded animal, extracting the homogenate with an acid, for instance, and subjecting the extract to a combination of chromatographic procedures such as reversed-phase chromatography, ion-exchange chromatography, affinity chromatography, etc.

As mentioned above, the ligand polypeptide in the present invention can be produced by the per se known procedures for peptide synthesis. The methods for peptide synthesis may be any of a solid-phase synthesis and a liquid-phase synthesis. Thus, the objective peptide can be produced by condensing a partial peptide or amino acid capable of constituting the protein with the residual part thereof and, when the product has a protective group, the protective group is detached whereupon a desired peptide can be manufactured. The known methods for condensation and deprotection includes the procedures described in the following literature (1)-(5).

(1) M. Bodanszky and M. A. Ondetti, Peptide Synthesis, Interscience Publishers, New York, 1966

(2) Schroeder and Luebke, The Peptide, Academic Press, New York, 1965

(3) Nobuo Izumiya et al., Fundamentals and Experiments in Peptide Synthesis, Maruzen, 1975

(4) Haruaki Yajima and Shumpei Sakakibara, Biochemical Experiment Series 1, Protein Chemistry IV, 205, 1977

(5) Haruaki Yajima (ed.), Development of Drugs-Continued, 14, Peptide Synthesis, Hirokawa Shoten

After the reaction, the protein can be purified and isolated by a combination of conventional purification techniques such as solvent extraction, column chromatography, liquid chromatography, and recrystallization. Where the protein isolated as above is a free compound, it can be converted to a suitable salt by the known method. Conversely where the isolated product is a salt, it can be converted to the free peptide by the known method.

The amide of polypeptide can be obtained by using a resin for peptide synthesis which is suited for

amidation. The resin includes chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethylmethylphenylacetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, 4-(2',4'-dimethoxyphenyl-Fmoc aminoethyl)phenoxy resin, and so on. Using such a resin, amino acids whose α-amino groups and functional groups of side-chain have been suitably protected are condensed on the resin according to the sequence of the objective peptide by various condensation techniques which are known per se. At the end of the series of reactions, the peptide or the protected peptide is removed from the resin and the protective groups are removed to obtain the objective polypeptide.

For the condensation of the above-mentioned protected amino acids, a variety of activating reagents for peptide synthesis can be used but a carbodiimide compound is particularly suitable. The carbodiimide includes DCC, N,N'-diisopropylcarbodiimide, and N-ethyl-N'-(3-dimethylaminoprolyl)carbodiimide. For activation with such a reagent, a racemization inhibitor additive, e.g. HOBt and the protected amino acid are directly added to the resin or the protected amino acid pre-activated as symmetric acid anhydride, HOBt ester, or HOOBt ester is added to the resin. The solvent for the activation of protected amino acids or condensation with the resin can be properly selected from among those solvents which are known to be useful for peptide condensation reactions. For example, N,N-dimethylformamide, N-methylpyrrolidone, chloroform, trifluoroethanol, dimethyl sulfoxide, DMF, pyridine, dioxane, methylene chloride, tetrahydrofuran, acetonitrile, ethyl acetate, or suitable mixtures of them can be mentioned. The reaction temperature can be

selected from the range hitherto-known to be useful for peptide bond formation and is usually selected from the range of about -20°C - 50°C. The activated amino acid derivative is generally used in a proportion of 1.5-4 fold excess. If the condensation is found to be insufficient by a test utilizing the ninhydrin reaction, the condensation reaction can be repeated to achieve a sufficient condensation without removing the protective group. If repeated condensation still fails to provide a sufficient degree of condensation, the unreacted amino group can be acetylated with acetic anhydride or acetylimidazole.

The protecting group of amino group for the starting material amino acid includes Z, Boc, tertiary-amyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthalyl, formyl, 2-nitrophenylsulfenyl, diphenylphosphinothioyl, or Fmoc. The carboxy-protecting group that can be used includes but is not limited to the above-mentioned C_{1-6} alkyl, C_{3-8} cycloalkyl and C_{7-14} aralkyl as well as 2-adamantyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, phenacyl, benzyloxycarbonylhydrazido, tertiary-butoxycarbonylhydrazido, and tritylhydrazido.

The hydroxy group of serine and threonine can be protected by esterification or etherification. The group suited for said esterification includes carbon-derived groups such as lower alkanoyl groups, e.g. acetyl etc., aroyl groups, e.g. benzoyl etc., benzyloxycarbonyl, and ethoxycarbonyl. The group suited for said etherification includes benzyl, tetrahydropyranyl, and tertiary-butyl.

The protective group for the phenolic hydroxyl group of tyrosine includes Bzl, Cl_2-Bzl, 2-nitrobenzyl, Br-Z, and tertiary-butyl.

The protecting group of imidazole for histidine

includes Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, and Fmoc.

The activated carboxyl group of the starting amino acid includes the corresponding acid anhydride, azide, and active esters, e.g. esters with alcohols such as pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccinimide, N-hydroxyphthalimide, HOBt, etc. The activated amino group of the starting amino acid includes the corresponding phosphoramide.

The method for elimination of protective groups includes catalytic reduction using hydrogen gas in the presence of a catalyst such as palladium black or palladium-on-carbon, acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid, trifluoroacetic acid, or a mixture of such acids, base treatment with diisopropylethylamine, triethylamine, piperidine, piperazine, reduction with sodium metal in liquid ammonia. The elimination reaction by the above-mentioned acid treatment is generally carried out at a temperature of -20°C - 40°C and can be conducted advantageously with addition of a cation acceptor such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethyl sulfide, 1,4-butanedithiol, 1,2-ethanedithiol. The 2,4-dinitrophenyl group used for protecting the imidazole group of histidine can be eliminated by treatment with thiophenol, while the formyl group used for protecting the indole group of tryptophan can be eliminated by alkali treatment with dilute sodium hydroxide solution or dilute aqueous ammonia as well as the above-mentioned acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol.

The method for protecting functional groups which should not take part in the reaction of the starting material, the protective groups that can be used, the

method of removing the protective groups, and the method of activating the functional groups that are to take part in the reaction can all be selected judicially from among the known groups and methods.

An another method for obtaining the amide form of the polypeptide comprises amidating the α-carboxyl group of the C-terminal amino acid at first, then extending the peptide chain to the N-side until the desired chain length, and then selectively deprotecting the α-amino group of the C-terminal peptide and the α-carboxy group of the amino acid or peptide that is to form the remainder of the objective polypeptide and condensing the two fragments whose α-amino group and side-chain functional groups have been protected with suitable protective groups mentioned above in a mixed solvent such as that mentioned hereinbefore. The parameters of this condensation reaction can be the same as described hereinbefore. From the protected peptide obtained by condensation, all the protective groups are removed by the above-described method to thereby provide the desired crude peptide. This crude peptide can be purified by known purification procedures and the main fraction be lyophilized to provide the objective amidated polypeptide.

To obtain an ester of the polypeptide, the α-carboxyl group of the C-terminal amino acid is condensed with a desired alcohol to give an amino acid ester and then, the procedure described above for production of the amide is followed.

The partial peptide of the ligand polypeptide of the present invention, its amide or ester, or a salt thereof can be any peptide that has the same activities, e.g. pituitary function modulating activity, central nervous system function modulating activity, or pancreatic function modulating activity as the polypeptide which has an amino acid sequence of SEQ

ID NO:73 or its substantial equivalent thereto. As such peptides, there can be mentioned peptides wherein 1 to 15 amino acids residues are deleted from the above-mentioned amino acid sequence of SEQ ID NO:73. To be specific, the peptide having an amino acid sequence corresponding to the 2nd to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 3rd to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 4th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 5th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 6th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 7th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 8th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 9th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 10th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 11th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 12th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 13th to 21st positions of the amino acid sequence of SEQ ID NO:73, the peptide corresponding to the 14th to 21st positions of the amino acid sequence of SEQ ID NO:73, and the peptide corresponding to the 15th to 21st positions of the amino acid sequence of SEQ ID NO:73, can be mentioned as preferred examples. Moreover, the peptide having the amino acid sequence of SEQ ID NO:74 is also preferred.

The ligand polypeptide or partial peptide thereof

can be used as antigen for preparation of anti-ligand polypeptide antibody. The polypeptide as antigen includes N-terminus peptides, C-terminus peptides or peptides of central portions other than above-mentioned ligand polypeptides or partial peptides thereof. To be more specifically includes the partial peptide of SEQ ID NO: 92, 93 or 94.

The partial peptide may be a peptide containing each of the domains or a peptide containing a plurality of the domains within the molecule.

The partial peptide mentioned in this specification may be one ending with an amide bond (-$CONH_2$) or an ester bond (-COOR) at the C-terminus. The ester here includes the same one of the above polypeptide. When the partial peptide has a carboxyl or carboxylate group in any position other than the C-terminus, the case in which such group or moiety has been amidated or esterified also falls within the scope of the partial peptide in the present invention. The ester here may be of the same one as the above-mentioned ester at the C-terminus.

The ligand polypeptide or its partial peptide of the present invention may be in the form of a fused protein which fused with a protein whose functions or properties are already known.

The salt of such partial peptide of the ligand polypeptide of present invention may be of the same one as the above-mentioned salt of the polypeptide.

The partial peptide of the ligand polypeptide of the invention, its amide or ester, or a salt thereof can be produced by the same synthetic processes as mentioned for the polypeptide or by cleaving the polypeptide of the present invention with a suitable peptidase.

The DNA coding for the ligand polypeptide or a partial peptide thereof of the present invention may be

any DNA comprising the nucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO:73 or its substantial equivalent thereto. Furthermore, the DNA may be any of genomic DNA, genomic DNA library, tissue- or cell-derived cDNA, tissue- or cell-derived cDNA library, and synthetic DNA. The vector for such as library may be any of bacteriophage, plasmide, cosmide, and phagimide. Moreover, it can be directly amplified by the RT-PCR method by using an RNA fraction may be prepared from a tissue or cells .

To be more specific, as the DNA coding for a polypeptide derived from rat whole brain or bovine hypothalmus and comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:44, the DNA comprising the nucleotide sequence of SEQ ID NO:2 can be exemplified. In SEQ ID NO:2, R at 129th position represents G or A, and Y at 179th and 240th positions represents C or T. When Y at 179th position is C, the amino acid sequence of SEQ ID NO:1 is encoded, and when Y at 179th position is T, the amino acid sequence of SEQ ID NO:44 is encoded.

As the DNA coding for a bovine-derived polypeptide comprising the amino acid sequence of SEQ ID NO:3, 4, 5, 6, 7, 8, 9 or 10, a DNA comprising the nucleotide sequence of SEQ ID NO:11, 12, 13, 14, 15, 16, 17 or 18 can be exemplified. Here, R at 63th position of SEQ ID NO:11, 13, 14 or 15 and R at 29th position of SEQ ID NO:12, 16, 17, or 18 represent G or A.

As the DNA coding for a rat-derived polypeptide of SEQ ID NO:45, 47, 48, 49, 50, 51, or 52, a DNA comprising the nucleotide sequence of SEQ ID NO:46, 53, 54, 55, 56, 57, or 58 can be exemplified.

Furthermore, as the DNA coding for a human-derived peptide of SEQ ID NO:59, 61, 62, 63, 64, 65, or 66, a DNA comprising the nucleotide sequence of SEQ ID NO:60, 67, 68, 69, 70, 71, or 72 can be exemplified.

Among DNAs coding for the bovine-derived polypeptide comprising the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:44, the rat-derived polypeptide comprising the amino acid sequence of SEQ ID NO:45, or the human-derived polypeptide comprising the amino acid sequence of SEQ ID NO:59, DNA fragments comprising partial nucleotide sequences of 6 to 90, preferably 6 to 60, more preferably 9 to 30, and especially preferably 12 to 30 can be advantageously used as DNA probes as well.

The DNA coding for the ligand polypeptide or a partial peptide thereof of the present invention can be produced by the following genetic engineering procedures.

The DNA fully encoding the polypeptide or partial peptide of the present invention can be cloned either by PCR amplification using synthetic DNA primers having a partial nucleotide sequence of the polypeptide or partial peptide or by hybridization using the DNA inserted in a suitable vector and labeled with a DNA fragment comprising a part or full region of a human-derived polypeptide or a synthetic DNA. The hybridization can be carried out typically by the procedure described in Molecular Cloning (2nd ed., J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). When a commercial library is used, the instructions given in the accompanying manual can be followed.

The cloned DNA coding for the polypeptide or partial peptide can be used directly or after digestion with a restriction enzyme or addition of a linker depending on purposes. This DNA has ATG as the translation initiation codon at the 5' end and may have TAA, TGA, or TAG as the termination codon at the 3' end. The translation initiation and termination codons can be added by means of suitable DNA adapters.

An expression vector for the polypeptide or

partial peptide can be produced by, for example (a) cutting out a target DNA fragment from the DNA for the polypeptide or partial peptide of the present invention and (b) ligating the target DNA fragment with the downstream side of a promoter in a suitable expression vector.

The vector may include plasmids derived from Escherichia coli, e.g., pBR322, pBR325, pUC12, pUC13, etc.; plasmids derived from Bacillus subtilis, e.g., pUB110, pTP5, pC194, etc.; plasmids derived from yeasts e.g., pSH19, pSH15, etc.; bacteriophages such as λ - phage, and animal virus such as retrovirus, vaccinia virus and baculovirus.

According to the present invention, any promoter can be used as long as it is compatible with the host cell which is used for expressing a gene. When the host for the transformation is E. coli, the promoters are preferably trp promoters, lac promoters, recA promoters, λ_{PL} promoters, lpp promoters, etc. When the host for the transformation is Bacillus, the promoters are preferably SPO1 promoters, SPO2 promoters, penP promoters, etc. When the host is a yeast, the promoters are preferably PHO5 promoters, PGK promoters, GAP promoters, ADH promoters, etc. When the host is an animal cell, the promoters include SV40-derived promoters, retrovirus promoters, metallothionein promoters, heat shock promoters, cytomegalovirus (CMV) promoters, SRα promoters, etc. An enhancer can be effectively utilized for expression.

As required, furthermore, a host-compatible signal sequence is added to the N-terminal side of the polypeptide or partial peptide thereof. When the host is E. coli, the utilizable signal sequences may include alkaline phosphatase signal sequences, OmpA signal sequences, etc. When the host is Bacillus, they may include α -amylase signal sequences, subtilisin signal

sequences, etc. When the host is a yeast, they may include mating factor α signal sequences, invertase signal sequences, etc. When the host is an animal cell, they may include insulin signal sequences, α -interferon signal sequences, antibody molecule signal sequences, etc.

A transformant or transfectant is produced by using the vector thus constructed, which carries the polypeptide or partial peptide-encoding DNA of the present invention. The host may be, for example, Escherichia microorganisms, Bacillus microorganisms, yeasts, insect cells, animal cells, etc. Examples of the Escherichia and Bacillus microorganisms include Escherichia coli K12·DH1 [Proc. Natl. Acad. Sci. USA, Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of molecular Biology, Vol, 41, 459 (1969)], C600 [Genetics, Vol. 39, 440 (1954)], etc.

Examples of the Bacillus microorganism are, for example Bacillus subtilis MI114 [Gene, Vol. 24, 255 (1983)], 207-21 [Journal of Biochemistry, Vol. 95, 76 (1984)], etc.

The yeast may be, for example, Saccharomyces cerevisiae AH22, AH22R⁻, NA87-11A, DKD-5D, 20B-12, etc. The insect may include a silkworm (Bombyx mori larva), [Maeda et al, Nature, Vol. 315, 592 (1985)] etc. The host animal cell may be, for example, monkey-derived cell line, COS-7, Vero, Chinese hamster ovary cell line (CHO cell), DHFR gene-deficient Chinese hamster cell line (dhfr⁻ CHO cell), mouse L cell, mouse myeloma cell, human FL, etc.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. Transformation of Escherichia microorganisms can be carried out in accordance with methods as

disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 69, 2110 (1972), Gene, Vol. 17, 107 (1982), etc. Transformation of Bacillus microorganisms can be carried out in accordance with methods as disclosed in, for example, Molecular & General Genetics, Vol. 168, 111 (1979), etc. Transformation of the yeast can be carried out in accordance with methods as disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 75, 1929 (1978), etc. The insect cells can be transformed in accordance with methods as disclosed in, for example, Bio/Technology, 6, 47-55, 1988. The animal cells can be transformed by methods as disclosed in, for example, Virology, Vol. 52, 456, 1973, etc. The transformants or transfectants wherein the expression vector carrying a polypeptide or partial peptide thereof encoding DNA harbors are produced according to the aforementioned techniques.

Cultivation of the transformant (transfectant) in which the host is Escherichia or Bacillus microorganism can be carried out suitably in a liquid culture medium. The culture medium may contains carbon sources, nitrogen sources, minerals, etc. necessary for growing the transformant. The carbon source may include glucose, dextrin, soluble starch, sucrose, etc. The nitrogen source may include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, etc. Examples of the minerals may include calcium chloride, sodium dihydrogen phosphate, magnesium chloride, etc. It is further allowable to add yeasts, vitamines, growth-promoting factors, etc. It is desired that the culture medium is pH from about 5 to about 8.

The Escherichia microorganism culture medium is preferably an M9 medium containing, for example, glucose and casamino acid (Miller, Journal of

Experiments in Molecular Genetics), 431-433, Cold Spring Harbor Laboratory, New York, 1972. Depending on necessity, the medium may be supplemented with drugs such as 3β -indolyl acrylic acid in order to improve efficiency of the promoter. In the case of an Escherichia host, the cultivation is carried out usually at about 15 to 43°C for about 3 to 24 hours. As required, aeration and stirring may be applied. In the case of Bacillus host, the cultivation is carried out usually at about 30 to 40°C for about 6 to 24 hours. As required, aeration and stirring may be also applied. In the case of the transformant in which the host is a yeast, the culture medium used may include, for example, a Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)], an SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)], etc. It is preferable that the pH of the culture medium is adjusted to be from about 5 to about 8. The cultivation is carried out usually at about 20 to 35°C for about 24 to 72 hours. As required, aeration and stirring may be applied. In the case of the transformant in which the host is an insect, the culture medium used may include those obtained by suitably adding additives such as passivated (or immobilized) 10% bovine serum and the like to the Grace's insect medium (Grace, T.C.C., Nature, 195, 788 (1962)). It is preferable that the pH of the culture medium is adjusted to be about 6.2 to 6.4. The cultivation is usually carried out at about 27°C for about 3 to 5 days. As desired, aeration and stirring may be applied. In the case of the transformant in which the host is an animal cell, the culture medium used may include MEM medium [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [Journal of the American

Medical Association, Vol. 199, 519 (1967)], 199 medium [Proceedings of the Society of the Biological Medicine, Vol. 73, 1 (1950)], etc. which are containing, for example, about 5 to 20% of fetal calf serum. It is preferable that the pH is from about 6 to about 8. The cultivation is usually carried out at about 30 to 40°C for about 15 to 60 hours. As required, medium exchange, aeration and stirring may be applied.

Separation and purification of the polypeptide or partial peptide from the above-mentioned cultures can be carried out according to methods described herein below.

To extract polypeptide or partial peptide from the cultured microorganisms or cells, the microorganisms or cells are collected by known methods after the cultivation, suspended in a suitable buffer solution, disrupted by ultrasonic waves, lysozyme and/or freezing and thawing, etc. and, then, a crude extract of the polypeptide or partial peptide is obtained by centrifugation or filtration. Other conventional extracting or isolating methods can be applied. The buffer solution may contain a protein-denaturing agent such as urea or guanidine hydrochloride or a surfactant such as Triton X-100 (registered trademark, hereinafter often referred to as "TM").

In the case where the polypeptide or partial peptide are secreted into culture media, supernatant liquids are separated from the microorganisms or cells after the cultivation is finished and the resulting supernatant liquid is collected by widely known methods. The culture supernatant liquid and extract containing the polypeptide or partial peptide can be purified by suitable combinations of widely known methods for separation, isolation and purification. The widely known methods of separation, isolation and purification may include methods which utilizes

solubility, such as salting out or sedimentation with solvents methods which utilizes chiefly a difference in the molecular size or weight, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in the electric charge, such as ion-exchange chromatography, methods utilizing specific affinity such as affinity chromatography, methods utilizing a difference in the hydrophobic property, such as reverse-phase high-performance liquid chromatography, and methods utilizing a difference in the isoelectric point such as isoelectric electrophoresis, or chromatofocusing, etc.

In cases where the polypeptide or partial peptide thus obtained is in a free form, the free protein can be converted into a salt thereof by known methods or method analogous thereto. In case where the polypeptide or partial peptide thus obtained is in a salt form vice versa, the protein salt can be converted into a free form or into any other salt thereof by known methods or method analogous thereto.

The polypeptide or partial peptide produced by the transformant can be arbitrarily modified or a polypeptide can be partly removed therefrom, by the action of a suitable protein-modifying enzyme before or after the purification. The protein-modifying enzyme may include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, etc. The activity of the polypeptide or partial peptide thus formed can be measured by experimenting the coupling (or binding) with receptor or by enzyme immunoassays (enzyme linked immunoassays) using specific antibodies.

The DNA coding for the ligand polypeptide of the present invention, the ligand polypeptide or a partial peptide thereof can be used for (1) synthesis of a part or the full length of the ligand for G protein-coupled

receptor protein, (2) search for the physiological activities of the ligand polypeptide or partial peptide thereof of the present invention, (3) preparation of a synthetic oligonucleotide probe or a PCR primer, (4) acquisition of DNAs coding for ligands of G protein-coupled receptor proteins and precursor proteins, (5) development of receptor-binding assay systems using the expression of recombinant receptor proteins and screening of candidate medicinally active compounds, (6) acquisition of antibodies and antisera, (7) development of diagnostic agents utilizing said antibodies or antisera, (8) development of drugs such as pituitary function modulators, central nervous system function modulators, and pancreatic function modulators, and (9) gene therapies, among other uses.

Particularly by using the receptor binding assay system using the expression of a recombinant G protein-coupled receptor protein, which is described hereinafter, agonists or antagonists of G protein-coupled receptors which are specific to warm-blood animals including humans can be screened and such agonists and antagonists can be used as prophylactic and therapeutic agents for various diseases.

Further, referring to (8) above, the ligand polypeptide, a partial peptide thereof, or the DNA encording either of them of the present invention is useful as a safe pharmaceutical composition of low toxic potential because it is recognized as a ligand by the G protein-coupled receptor protein expressed in the hypophysis, central nervous system and pancreatic β cells. The ligand polypeptide, a partial peptide thereof, or the DNA encording either of them of the present invention is associated with the modulation of pituitary function, central nervous system function, and pancreatic function and, therefore, can be used as a therapeutic and prophylactic pharmaceutical

composition for dementia such as senile dementia, cerebrovascular dementia (dementia due to cerebrovascular disorder), dementia associated with phylodegenerative retroplastic diseases (e.g. Alzheimer's disease, Parkinson's disease, Pick's disease, Huntington's disease, etc.), dementia due to infectious diseases (e.g. delayed viral infections such as Creutzfelt-Jakob disease), dementia associated with endocrine, metabolic, and toxic diseases (e.g. hypothyroidism, vitamin B12 deficiency, alcoholism, and poisoning due to various drugs, metals, or organic compounds), dementia associated with oncogenous diseases (e.g. brain tumor), dementia due to traumatic diseases (e.g. chronic subdural hematoma):, depression (melancholia), hyperkinetic (microencephalo-pathy) syndrome, disturbance of consciousness, anxiety syndrome, schizophrenia, horror, growth hormone secretory disease (e.g. gigantism, acromegalic gigantism etc.), hyperphagia, polyphagia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, hyperprolactinemia, diabetes (e.g. diabetic complications, diabetic nephropathy, diabetic neurophathy, diabetic retinopathy etc.), cancer (e.g. mammary cancer, lymphatic leukemia, cystic cancer, ovary cancer, prostatic cancer etc.), pancreatitis, renal disease (e.g. chromic renal failure, nephritis etc.), Turner's syndrome, neurosis, rheumatoid arthritis, spinal injury, transient brain ischemia, amyotrophic lateral sclerosis, acute myocardial infarction, spinocerebellar degeneration, bone fracture, trauma, atopic dermatitis, osteoporosis, asthma, epilepsy, infertility or oligogalactia. Furthermore, they can be also used as the agent for improvement in postoperative nutritional status and/or vasopressor.

When the polypeptide, a partial peptide thereof,

or the DNA encoding either of them of the present invention is used as a pharmaceutical composition as described above, it can be used by conventional methods. For example, it can be used orally in the form of tablets which may be sugar coated as necessary, capsules, elixirs, microcapsules etc., or non-orally in the form of injectable preparations such as aseptic solutions and suspensions in water or other pharmaceutically acceptable liquids. These preparations can be produced by mixing the polypeptide, a partial peptide thereof, or the DNA encoding either of them with physiologically acceptable carriers, flavoring agents, excipients, vehicles, antiseptics, stabilizers, binders etc. in unit dosage forms required for generally accepted manners of pharmaceutical making. Active ingredient contents in these preparations are set so that an appropriate dose within the specified range is obtained.

Additives which can be mixed in tablets, capsules etc. include binders such as gelation, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint, akamono oil and cherry. When the unit dosage form is the capsule, the above-mentioned materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical making such as by dissolving or suspending active ingredients, naturally occuring vegetable oils such as sesame oil and coconut oil, etc. in vehicles such as water for injection.

Aqueous liquids for injection include physiological saline and isotonic solutions containing

glucose and other auxiliary agents, e.g., D-sorbitol, D-mannitol and sodium chloride, and may be used in combination with appropriate dissolution aids such as alcohols, e.g., ethanol, polyalcohols, e.g., propylene glycol and polyethylene glycol, nonionic surfactants, e.g., polysorbate 80 (TM) and HCO-50 etc. Oily liquids include sesame oil and soybean oil, and may be used in combination with dissolution aids such as benzyl benzoate and benzyl alcohol. Furthermore the above-mentioned materials may also be formulated with buffers, e.g., phosphate buffer and sodium acetate buffer; soothing agents, e.g., benzalkonium chloride, procaine hydrochloride; stabilizers, e.g., human serum albumin, polyethylene glycol; preservatives, e.g., benzyl alcohol, phenol; antioxidants etc. The thus-prepared injectable liquid is normally filled in an appropriate ampule. Because the thus-obtained preparation is safe and of low toxicity, it can be administered to humans or warm-blooded mammals, e.g., mouse, rats, guinea pig, rabbits, chicken, sheep, pigs, bovines, cats, dogs, monkeys, baboons, chimpanzees, for instance.

The dose of said polypeptide, a partial peptide thereof, or the DNA encoding either of them is normally about 0.1-100 mg, preferably 1.0-50 mg, and more preferably 1.0-20 mg per day for an adult (weighing 60 kg) in oral administration, depending on symptoms etc. In non-oral administration, it is advantageous to administer the polypeptide, a partial peptide thereof, or the DNA encoding either of them in the form of injectable preparation at a daily dose of about 0.01-30 mg, preferably about 0.1-20 mg, and more preferably about 0.1-10 mg per administration by an intravenous injection for an adult (weighing 60 kg), depending on subject of administration, target organ, symptoms, method of administration etc. For other animal

species, corresponding does as converted per 60 kg weight can be administered.

The G protein-coupled receptor protein for the above ligand polypeptide of the present invention may be any of G protein-coupled receptor proteins derived from various tissues, e.g. hypophysis, pancreas, brain, kidney, liver, gonad, thyroid gland, gall bladder, bone marrow, adrenal gland, skin, muscle, lung, alimentary canal, blood vessel, heart, etc. of human and other warm-blooded animals, e.g. guinea pig, rat, mouse, swine, sheep, bovine, monkey, etc.; and comprising an amino acid sequence of SEQ ID NO:19, 20, 21, 22 or 23, or substantial equivalent thereto. Thus, the G protein-coupled receptor protein of the present invention includes, in addition to proteins comprising the SEQ ID NO:19, 20, 21, 22 or 23, those proteins comprising amino acid sequences of about 90-99.9% homology to the amino acid sequence of SEQ ID NO:19, 20, 21, 22 or 23 and having qualitatively substantially equivalent activity to proteins comprising the amino acid sequence of SEQ ID NO:19, 20, 21, 22, or 23. The activities which these proteins are possessed may include ligand binding activity and signal transduction activity. The term "substantially equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as the strength of ligand binding activity and the molecular weight of receptor protein are present.

To be further specific, the G protein-coupled receptor proteins include human pituitary-derived G protein-coupled receptor proteins which comprises the amino acid sequence of SEQ ID NO:19 or/and SEQ ID NO:20, mouse pancreas-derived G protein-coupled receptor proteins which comprises the amino acid sequence of SEQ ID NO:22, and mouse pancreas-derived G

protein-coupled receptor proteins which comprises the amino acid sequence of SEQ ID NO:23. As the human pituitary-derived G protein-coupled receptor proteins which comprises the amino acid sequence of SEQ ID NO:19 and/or SEQ ID NO:20 include the human pituitary-derived G protein-coupled receptor protein which comprises the amino acid sequence of SEQ ID NO:21. The G protein-coupled receptor proteins further include proteins wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are deleted from the amino acid sequence of SEQ ID NO:19, 20, 21, 22 or 23, proteins wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues are added to the amino acid sequence of SEQ ID NO:19, 20, 21, 22, or 23, the proteins wherein 1 to 30 amino acid residues, preferably 1 to 10 amino acid residues in the amino acid sequence of SEQ ID NO:19, 20, 21, 22, or 23 are substituted with one or more other amino acid residues.

Here, the protein which comprises an amino acid sequence of SEQ ID NO:21 or substantial equivalent thereto contains the full-length of the amino acid sequence for human pituitary-derived G protein-coupled receptor protein. The protein which comprises an amino acid sequence of SEQ ID NO:19 or/and SEQ ID NO:20 or substantial equivalent thereto may be a partial peptide of the protein which comprises an amino acid sequence of SEQ ID NO:21 or substantial equivalent thereto. The protein which comprises an amino acid sequence of SEQ ID NO:22 or SEQ ID NO:23 or substantial equivalent thereto is a G protein-coupled receptor protein which is derived from mouse pancreas but since its amino acid sequence is quite similar to the amino acid sequence of SEQ ID NO:19 or/and SEQ ID NO:20 (cf. Example 8, Fig. 13 in particular), the protein which comprises an amino acid sequence of SEQ ID NO:22 or 23 or substantial equivalent thereto is also subsumed in the category of

said partial peptide of the protein which comprises an amino acid sequence of SEQ ID NO:21 or substantial equivalent thereto.

Thus, the above-mentioned protein comprising an amino acid sequence of SEQ ID NO:21 or substantial equivalent thereto or a partial peptide of the protein or a salt thereof, which will be described below, includes the protein comprising an amino acid sequence of SEQ ID NO:19, 20, 22, or 23 or substantial equivalent thereto, or a salt thereof.

Furthermore, the G protein-coupled receptor protein includes the protein in which the N-terminal Met has been protected with a protective group, e.g. C_{1-6} acyl such as formyl or acetyl, the protein in which the N-terminal side of Glu has been cleaved in vivo to form pyroglutamine, the protein in which the side chain of any relevant constituent amino acid has been protected with a suitable protective group, e.g. C_{1-6} acyl such as formyl or acetyl, and the complex protein such as glycoproteins available upon attachment of sugar chains.

The salt of G protein-coupled receptor protein includes the same kinds of salts as mentioned for the ligand polypeptide.

The G protein-coupled receptor protein or a salt thereof or a partial peptide thereof can be produced from the tissues or cells of human or other warm-blooded animals by the per se known purification technology or, as described above, by culturing a transformant carrying a DNA coding for the G protein-coupled receptor protein. It can also be produced in accordance with the procedures for peptide synthesis which are described above.

A partial peptide of G protein-coupled receptor protein may include, for example, a fragment containing an extracellular portion of the G protein-coupled

receptor protein, i.e. the site which is exposed outside the cell membranes. Examples of the partial peptide are fragments containing a region which is an extracellular area (hydrophilic region) as analyzed in a hydrophobic plotting analysis of the G protein-coupled receptor protein, such as shown in Fig. 3, Fig. 4, Fig. 8, Fig. 11, or Fig. 14. Furthermore, a fragment which partly contains a hydrophobic region may also be used. While peptides which separately contains each domain may be used too, peptides which contains multiple domains at the same time will be used as well.

The salt of a partial peptide of G protein-coupled receptor protein may be the same one of salt mentioned for the salt of ligand polypeptide.

The DNA coding for the G protein-coupled receptor protein may be any DNA comprising a nucleotide sequence encoding the G protein-coupled receptor protein which comprises an amino acid sequence of SEQ ID NO:19, 20, 21, 22, or 23 or substantial equivalent thereto. It may also be any one of genomic DNA, genomic DNA library, tissue- or cell-derived cDNA, tissue- or cell-derived cDNA library, and synthetic DNA. The vector for such a library may include bacteriophage, plasmid, cosmid, and phargimide. Furthermore, using an RNA fraction prepared from a tissue or cells, a direct amplification can be carried out by the RT-PCR method.

To be specific, the DNA encoding the human pituitary-derived G protein-coupled receptor protein which comprises the amino acid sequence of SEQ ID NO:19 include a DNA which comprises the nucleotide sequence of SEQ ID NO:24. The DNA encoding the human pituitary-derived G protein-coupled receptor protein which comprises the amino acid sequence of SEQ ID NO:20 include a DNA which comprises the nucleotide sequence of SEQ ID NO:25. The DNA encoding the human pituitary-derived G protein-coupled receptor protein which

comprises the amino acid sequence of SEQ ID NO:21 include a DNA which comprises the nucleotide sequence of SEQ ID NO:26. The DNA encoding the mouse pancreas-derived G protein-coupled receptor protein which comprises the amino acid sequence of SEQ ID NO:22 include a DNA which comprises the nucleotide sequence of SEQ ID NO:27. The DNA encoding the mouse pancreas-derived G protein-coupled receptor protein which comprises the amino acid sequence of SEQ ID NO:23 include a DNA comprising the nucleotide sequence of SEQ ID NO:28.

A method for cloning the DNA completely coding for the G protein-coupled receptor protein, vector, promoter, host cell, a method for transformation, a method for culturing the transformant or a method for separation and purification of the G protein-coupled receptor protein may include the same one as mentioned for the ligand polypeptide.

To be specific, the plasmid phGR3 obtained in Example 5, described hereinafter, is digested with the restriction enzyme SalI and the translation frame for the full-length cDNA encoding hGR3 is isolated. This frame is subjected to ligation to, for example, the expression vector pAKKO-111 for animal cell use which has been treated with BAP (bacterial alkaline phosphatase) after SalI digestion for inhibition of autocyclization. After completion of the ligation reaction, a portion of the reaction mixture is used for transfection of, for example, Escherichia coli DH5. Among the transformants obtained, a transformant in which the cDNA coding for hGR3 has been inserted in the forward direction with respect to a promoter, such as SRα, which has been inserted into the expression vector beforehand is selected by mapping after cleavage with restriction enzymes or by nucleotide sequencing and the plasmid DNA is prepared on a production scale.

The thus-constructed DNA of the expression vector is introduced into CHO dhfr$^-$ cells using a kit for introducing a gene into animal cells by the calcium phosphate method, the liposome method or the like to provide a high G protein-coupled receptor protein (hGR3) expression CHO cell line.

The resulting CHO cells are cultured in a nucleic acid-free screening medium in a CO_2 incubator at 37°C using 5% CO_2 for 1-4 days so as to give the G protein-coupled receptor protein (hGR3).

The G protein-coupled receptor protein is purified from the above CHO cells using an affinity column prepared by conjugating an antibody to the G protein-coupled receptor protein or a partial peptide thereof to a support or an affinity column prepared by conjugating a ligand for the G protein-coupled receptor protein.

The activity of the G protein-coupled receptor protein thus formed can be measured by experimenting the binding with a ligand or by enzyme immunoassays using specific antibodies.

The G protein-coupled receptor protein, the partial peptide thereof and the G protein-coupled receptor protein-encoding DNA can be used for:

1) determining a ligand to the G protein-coupled receptor protein,

2) obtaining an antibody and an antiserum,

3) constructing a system for expressing a recombinant receptor protein,

4) developing a receptor-binding assay system using the above developing system and screening pharmaceutical candidate compounds,

5) designing drugs based upon comparison with ligands and receptors which have a similar or analogous structure,

6) preparing a probe for the analysis of genes and

preparing a PCR primer,

7) gene manipulation therapy,

In particular, it is possible to screen a G protein-coupled receptor agonist or antagonist specific to a warm-blooded animal such as human being by a receptor-binding assay system which uses a system for expressing a recombinant G protein-coupled receptor protein. The agonist or antagonist thus screened or characterized permits various applications including prevention and/or therapy of a variety of diseases.

Described below are uses of ligand polypeptide of the present invention, G protein-coupled receptor proteins to the ligand polypeptide, ligand polypeptide-encoding DNAs, G protein-coupled receptor protein-encoding DNAs and their antibodies.

(1) Method for Determing a Ligand to the G protein-coupled receptor Protein

The G protein-coupled receptor protein, the partial peptide thereof or a salt thereof is useful as a reagent for investigating or determining a ligand to said G protein-coupled receptor protein.

According to the present invention, methods for determining a ligand to the G protein-coupled receptor protein which comprises contacting the G protein-coupled receptor protein or the partial peptide thereof with the compound to be tested, and measuring the binding amount, the cell stimulating activity, etc. of the test compound to the G protein-coupled receptor protein or the partial peptide thereof are provided.

The compound to be tested may include not only known ligands such as angiotensins, bombesins, canavinoids, cholecystokinins, glutamine, serotonin, melatonins, neuropeptides Y, opioids, purine, vasopressins, oxytocins, VIP (vasoactive intestinal and related peptides), somatostatins, dopamine, motilins, amylins, bradykinins, CGRP (calcitonin gene related

peptides), leukotrienes, pancreastatins, prostaglandins, thromboxanes, adenosine, adrenaline, α- and β- chemokines such as IL-8, GROα, GROβ, GROγ, NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1α, MIP-1β, RANTES, etc.; endothelins, enterogastrins, histamine, neurotensins, TRH, pancreatic polypeptides, galanin, modified derivatives thereof, analogues thereof, family members thereof and the like but also tissue extracts, cell culture supernatants, etc. of human or warm-blooded aminals such as mice, rats, swines, cattle, sheep and monkeys, etc. For example, said tissue extract, said cell culture supernatant, etc. is added to the G protein-coupled receptor protein for measurement of the cell stimulating activity, etc. and fractionated by relying on the measurements whereupon a single ligand can be finally determined and obtained.

In one specific embodiment of the present invention, said method for determining the ligand includes a method for determining whether a sample (including a compound or a salt thereof) is capable of stimulating a target cell which comprises binding said compound with the G protein-coupled receptor protein either int he presence of the G protein-coupled receptor protein, the partial peptide thereof or a salt thereof, or in a receptor binding assay system in which the expression system for the recombinant receptor protein is constructed and used; and measuring the receptor-mediated cell stimulating activity, etc. Examples of said cell stimulating activities that can be measured include promoting or inhibiting biological responses, e.g. liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+}, production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation

of endocellular protein, activation of c-fos, decrease in pH, etc, and preferably liberation of arachidonic acid. Examples of said compound or a salt thereof capable of stimulating the cell via binding with the G protein-coupled receptor protein include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermented products, etc.

In more specific embodiments of the present invention, said methods for screening and identifying a ligand includes:

1) a method of screening for a ligand to a G protein-coupled receptor protein, which comprises contacting a labeled test compound with a G protein-coupled receptor protein or a salt thereof or its partial peptide or a salt thereof, and measuring the amount of the labeled test compound binding with said protein or salt thereof or with said partial peptide or salt thereof;

2) a method of screening for a ligand to a G protein-coupled receptor protein, which comprises contacting a labeled test compound with cells containing the G protein-coupled receptor protein or the membrane fraction of said cell, and measuring the amount of the labeled test compound binding with said cells or said membrane fraction;

3) a method of screening for a ligand to a G protein-coupled receptor protein, which comprises contacting a labeled test compound with the G protein-coupled receptor protein expressed on cell membranes by culturing transformants carrying the G protein-coupled receptor protein-encoding DNA and measuring the amount of the labeled test compound binding with said G protein-coupled receptor protein;

4) a method of screening for a ligan to a G protein-coupled receptor protein, which comprises contacting a test compound with cells containing the G protein-coupled receptor protein, and measuring the cell

stimulating activity, e.g. promoting or inhibiting activity on biological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+}, production of endocullular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, etc. via the G protein-coupled receptor protein; and

5) a method of screening for a ligand to the G protein-coupled receptor protein, which comprises contacting a test compound with the G protein-coupled receptor protein expressed on the cell membrane by culturing transformants carrying the G protein-coupled receptor protein-encoding DNA, and measuring at least one cell stimulating activity, e.g., an activity for promoting or inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+}, production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH etc. via the G protein-coupled receptor protein.

Described below are specific illustrations of the method for screening and identifying ligands.

First, the G protein-coupled receptor protein used for the method for determining the ligand may include any material so far as it contains a G protein-coupled receptor protein, a partial peptide thereof or a salt thereof although it is preferable to express large amounts of the G protein-coupled receptor proteins in animal cells.

In the manufacture of the G protein-coupled receptor protein, the above-mentioned method can be used and carried out by expressing said protein

encoding DNA in mammalian cells or in insect cells. With respect to the DNA fragment coding for a particular region such as an extracellular epitope, the extracellular domains, etc., complementary DNA may be used although the method of expression is not limited thereto. For example, gene fragments or synthetic DNA may be used as well.

In order to introduce the G protein-coupled receptor protein-encoding DNA fragment into host animal cells and to express it efficiently, it is preferred that said DNA fragment is incorporated into the downstream side of polyhedron promoters derived from nuclear polyhedrosis virus belonging to baculovirus, promoters derived from SV40, promoters derived from retrovirus, metallothionein promoters, human heat shock promoters, cytomegalovirus promoters, SRα promoters, etc. Examinations of the quantity and the quality of the expressed receptor can be carried out by methods per se known to those of skill in the art or methods similar thereto based upon the present disclosure. For example, they may be conducted by methods described in publications such as Nambi, P. et al: The Journal of Biochemical Society, vol.267, pages 19555-19559 (1992).

Accordingly, with respect to the determination of the ligand, the material containing a G protein-coupled receptor protein or partial peptide thereof may include products containing G protein-coupled receptor proteins which are purified by methods per se known to those of skill in the art or methods similar thereto, peptide fragments of said G protein-coupled receptor protein, cells containing said G protein-coupled receptor protein, membrane fractions of the cell containing said protein, etc.

When the G protein-coupled receptor protein-containing cell is used in the determining method of the ligand, said cell may be immobilized with binding

agents including glutaraldehyde, formalin, etc. The immobilization may be carried out by methods per se known to those of skill in the art or methods similar thereto.

The G protein-coupled receptor protein-containing cells are host cells which express the G protein-coupled receptor protein. Examples of said host cells are microorganisms such as Escherichia coli, Bacillus subtilis, yeasts, insect cells, animal cells, etc.

The cell membrane fraction is a cell membrane-rich fraction which is prepared by methods per se known to those of skill in the art or methods similar thereto after disruption of cells. Examples of cell disruption may include a method for squeezing cells using a Potter-Elvehjem homogenizer, a disruption by a Waring blender or a Polytron manufactured by Kinematica, a disruption by ultrasonic waves, a disruption via blowing out cells from small nozzles together with applying a pressure using a French press or the like, etc. In the fractionation of the cell membrane, a fractionation method by means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation is mainly used. For example, disrupted cellular liquid is centrifuged at a low speed (500 rpm to 3,000 rom) for a short period (usually, from about one to ten minutes), the supernatant liquid is further centrifuged at a high speed (15,000 rpm to 30,000 rom) usually for 30 minutes to two hours and the resulting precipitate is used as a membrane fraction. Said membrane fraction contains a lot of the expressed G protein-coupled receptor protein and a lot of membrane components such as phospholipids and membrane proteins derived from the cells.

The amount of the G protein-coupled receptor protein in the membrane fraction cell containing said G protein-coupled receptor protein is preferably 10^3 to

10^8 molecules per cell or, more preferably, 10^5 to 10^7 molecules per cell. Incidentally, the greater the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system becomes possible and, moreover, it permits measurement of a large amount of samples within the same lot.

In conducting the above-mentioned methods 1) to 3) wherein ligands capable of binding with the G protein-coupled receptor protein are determined, a suitable G protein-coupled receptor fraction and a labeled test compound are necessary. The G protein-coupled receptor fraction is preferably a naturally occurring (natural type) G protein-coupled receptor, a recombinant G protein-coupled receptor having the activity equivalent to that of the natural type. Here, the term "activity equivalent to" means the equivalent ligand binding activity, etc. as discussed above.

Suitable examples of the labeled test compound include above-mentioned compound to be tested which are labeled with [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc.

Specifically, the determination of ligands capable of binding with G protein-coupled receptor proteins is carried out as follows:

First, cells or cell membrane fractions containing the G protein-coupled receptor protein are suspended in a buffer suitable for the assay to prepare the receptor sample for conducting the method of determining the ligand binding with the G protein-coupled receptor protein. The buffer may include any buffer such as Tris-HCL buffer or phosphate buffer with pH 4-10, preferably, pH 6-8, etc., as long as it does not inhibit the binding of the ligand with the receptor. In addition, surface-active agents such as CHAPS, Tween 80™ (Kao-Atlas, Japan), digitonin, deoxycholate, etc.

and various proteins such as bovine serum albumin (BSA), gelatin, milk derivatives, etc. may be added to the buffer with an object of descreasing the non-specific binding. Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A test compound labeled with a predetermined (or certain) amount (5,000 cpm to 500,000 cpm) of [^{3}H], [^{125}I]. [^{14}C], [^{35}S], etc. coexists in 0.01 ml to 10 ml of said receptor solution. In order to know the non-specific binding amount (NSB), a reaction tube to which a great excessive amount of the unlabeled test compound is added is prepared as well. The reaction is carried out at 0-50°C, preferably at 4-37°C for 20 minutes to 24 hours, preferably 30 minutes to three hours. After the reaction, it is filtered through a glass fiber filter or the like, washed with a suitable amount of the same buffer and the radioactivity remaining in the glass fiber filter is measured by means of a liquid scintillation counter or a gamma-counter. The test compound in which the cound (B - NSB) obtained by subtracting the non-specific binding amount (NSB) from the total binding amount (B) is more than 0 cpm is identified as a ligand to the G protein-coupled receptor protein.

In conducting the above-mentioned methods 4) to 5) wherein ligands capable of binding with the G protein-coupled receptor protein are determined, the cell stimulating activity, e.g. the liberation of arachidonic acid, the liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, the production of inositol phosphate, changes in the cell membrane potential, the phosphorylation of endocellular protein, the activation of c-fos, lowering of pH, the activation of G protein,

cell promulgation, etc.; mediated by the G protein-coupled receptor protein may be measured by known methods or by the use of commercially available measuring kits. To be more specific, G protein-coupled receptor protein-containing cells are at first cultured in a multi-well plate or the like.

In conducting the determination of ligand, it is substituted with a fresh medium or a suitable buffer which does not show toxicity to the cells in advance of the experiment, and incubated under appropriate conditions and for sufficient time after adding a test compound, etc. thereto. Then, the cells are extracted or the supernatant liquid is recovered and the resulting product is determined by each of the methods. When it is difficult to identify the production of the substance, e.g. arachidonic acid, etc. which is to be an index for the cell stimulating activity due to the decomposing enzyme contained in the cell, an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to an activity such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the production of the cells whose fundamental production is increased by forskolin or the like.

The kit used for the method of determining the ligand binding with the G protein-coupled receptor protein includes a G protein-coupled receptor protein or a partial peptide thereof, cells containing the G protein-coupled receptor protein, a membrane fraction from the cells containing the G protein-coupled receptor protein, etc.

Examples of the kit for determining the ligand are as follows:

1. Reagent for Determing the Ligand.

1) Buffer for Measurement and Buffer for Washing.

The buffering product wherein 0.05% of bovine

serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This product may be sterilized by filtration through a membrane filter with a 0.45 μm pore size, and stored at 4°C or may be formulated upon use.

2) G protein-coupled receptor Protein Sample.

CHO cells in which G protein-coupled receptor proteins are expressed are subcultured at the rate of 5 x 10^5 cells/well in a 12-well plate and cultured at 37°C in a humidified 5% CO_2/95% air atmosphere for two days to prepare the sample.

3) Labeled Test Compound.

The compound which is labeled with commercially available [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc. or labeled with a suitable method.

The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1 μM with a buffer for the measurement. In the case of a test compound which is barely soluble in water, it may be dissolved in an organic solvent such as dimethylformamide, DMSO, methanol and the like.

4) Unlabeled Test Compound.

The same compound as the labeled one is prepared in a concentration of 100 to 1,000-fold concentrated state.

2. Method of Measurement

1) G protein-coupled receptor protein-expressing CHO cells cultured in a 12-well tissue culture plate are washed twice with 1 ml of buffer for the measurement and then 490 μl of buffer for the measurement is added to each well.

2) Five μl of the labeled test compound is added and the mixture is made to react at room temperature for one hour. For measuring the nonspecific binding amount, 5μl of the unlabeled test compound is added.

3) The reaction solution is removed from each well,

which is washed with 1 ml of a buffer for the measurement three times. The labeled test compound which is binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A manufactured by WAKO Pure Chemical, Japan.

4) Radioactivity is measured using a liquid scintillation counter such as one manufactured by Beckmann.

(2) Prophylactic and Therapeutic Agent for G protein-coupled receptor Protein or Ligand Polypeptide Deficiency Diseases

If a ligand to the G protein-coupled receptor protein is revealed via the aforementioned method (1), the ligand or the G protein-coupled receptor protein-encoding DNA can be used as a prophylactic and/or therapeutic agent for treating said G protein-coupled receptor protein or ligand polypeptide deficiency diseases depending upon the action that said ligand exerts.

For example, when there is a patient for whom the physiological action of the ligand, e.g. pituitary function modulating action, central nervous system function modulating action or pancreatic function modulating action; cannot be expected because of a descrease in the G protein-coupled receptor protein or ligand polypeptide in vivo, the amount of the G protein-coupled receptor protein or ligand polypeptide in the brain cells of said patient can be increased whereby the action of the ligand can be fully achieved by:

(a) administering the G protein-coupled receptor protein-encoding DNA to the patient to express it; or

(b) inserting the G protein-coupled receptor protein or ligand polypeptide-encoding DNA into brain cells or the like to said patient. Accordingly, the G protein-

coupled receptor protein- or ligand polypeptide-encoding DNA can be used as a safe and less toxic preventive and therapeutic agent for the G protein-coupled receptor protein or ligand polypeptide deficiency diseases.

When the above-mentioned DNA is used as the above-mentioned agent, said DNA may be used alone or after inserting it into a suitable vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc. followed by subjecting the product vector to a conventional means which is the same means as using the DNA coding for the ligand polypeptide or partial peptide thereof as the pharmaceutical composition.

(3) Quantitative Determination of the G protein-coupled receptor Protein to the Ligand Polypeptide

The ligand polypeptide that has a binding property for a G protein-coupled receptor protein or a partial peptide thereof, or a salt thereof are capable of determining quantitatively an amount of a G protein-coupled receptor protein or a partial peptide thereof, or a salt thereof in vivo with good sensitivity.

This quantitative determination may be carried out by, for example, combining with a competitive analysis. Thus, a sample to be determined is contacted with the ligand polypeptide so that the concentration of a G protein-coupled receptor protein or a partial peptide thereof in said sample can be determined. In one embodiment of the quantitative determination, the protocols described in the following 1) and 2) or methods similar thereto may be used:

1) Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); and

2) Hiroshi Irie (ed): "Radioimmunoassay, Second Series" (Kodansha, Japan, 1979).

(4) Screening of Compound Changing the Binding

Activity of Ligand Polypeptide, Partial Peptide thereof or salt thereof (hereinafter sometimes referred to briefly as ligand or ligand polypeptide) with the G protein-coupled receptor Protein

G protein-coupled receptor proteins or partial peptide or salt thereof can be used. Alternatively, expression systems for recombinant G protein-coupled receptor proteins are constructed and receptor binding assay systems using said expression system are used. In these assay systems, it is possible to screen compounds, e.g. peptides, proteins, nonpeptidic compounds, synthetic compounds, formented products, cell extracts, animal tissue extracts, etc.; or salts thereof which changes the binding activity of a ligand polypeptide with the G protein-coupled receptor protein. Such a compound includes a compound exhibiting a G protein-coupled receptor-mediated cell stimulating activity, e.g. activity of promoting or activity of inhibiting physiological reactions including liberation of arachidonic acid, liberation of acetylchloline, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular protein,s activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.; so-called "G protein-coupled receptor-agonist", a compound free from such a cell stimulating activity, so-called "G protein coupled receotor-antagonist", etc. The term of "change the binding activity of a ligand polypeptide" includes the both concept of the case in which the binding of ligand is inhibited and the case in which the binding of ligand is promoted.

Thus, the present invention provides a method of screening for a compound which changes the binding

activity of a ligand with a G protein-coupled receptor protein or a salt thereof, characterized by comparing the following two cases:

(i) the case wherein the ligand is contacted with the G protein-coupled receptor protein or salt thereof, or a partial peptide thereof or a salt thereof; and

(ii) the case wherein the ligand is contacted with a mixture of the G protein-coupled receptor protein or salt thereof or the partial peptide or salt thereof and said test compound.

In said screening method, one characteristic feature of the present invention resides in that the amount of the ligand bonded with said G protein-coupled receptor protein or the partial peptide thereof, the cell stimulating activity of the ligand, etc. are measured in both the case where (i) the ligand polypeptide is contacted with G protein-coupled receptor proteins or partial peptide thereof and in the case where (ii) the ligand polypeptide and the test compound are contacted with the G protein-coupled receptor protein or the partial peptide thereof, respectively and then compared therebetween.

In one more specific embodiment of the present invention, the following is provided:

1) a method of screening for a compound or a salt thereof which changes the binding activity of a ligand polypeptide with a G protein-coupled receptor protein, characterized in that, when a labeled ligand polypeptide is contacted with a G protein-coupled receptor protein or a partial peptide thereof and when a labeled ligand polypeptide and a test compound are contacted with a G protein-coupled receptor protein or a partial peptide thereof, the amounts of the labeled ligand polypeptide bonded with said protein or a partial peptide thereof or a salt thereof are measured and compared;

2) a method of screening for a compound or a salt thereof which changes the binding activity of a ligand polypeptide with a G protein-coupled receptor protein, characterized in that, when a labeled ligand polypeptide is contacted with cells containing G protein-coupled receptor proteins or a membrane fraction of said cells and when a labeled ligand polypeptide and a test compound are contacted with cells containing G protein-coupled receptor proteins or a membrane fraction of said cells, the amounts of the labeled ligand polypeptide binding with said protein or a partial peptide thereof or a salt thereof are measued and compared;

3) a method of screening for a compound or a salt thereof which changes the binding activity of a ligand polypeptide with a G protein-coupled receptor protein, characterized in that, when a labeled ligand polypeptide is contacted with G protein-coupled receptor proteins expressed on the cell memberane by culturing a transformant carrying a G protein-coupled receptor protein-encoding DNA and when a labeled ligand polypeptide and a test compound are contacted with G protein-coupled receptor proteins expressed on the cell membrane by culturing a transformant carrying a G protein-coupled receptor protein-encoding DNA, the amounts of the labeled ligand polypeptide binding with said G protein-coupled receptor protein are measured and compared;

4) a method of screening for a compound or a salt thereof which changes the binding of a ligand polypeptide with a G protein-coupled receptor protein, characterized in that, when a G protein-coupled receptor protein-activating compound, e.g. a ligand polypeptide of the present invention, etc. is contacted with cells containing G protein-coupled receptor proteins and when the G protein-coupled receptor

protein-activating compound and a test compound are contacted with cells containing G protein-coupled receptor proteins, the resulting G protein-coupled receptor protein-mediated cell stimulting activities, e.g. activities of promoting or activities of inhibiting physiological responses including liberation of arachidonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.; are measured and compared; and

5) a method of screening for a compound or a salt thereof which changes the binding activity of a ligand polypeptide with a G protein-coupled receptor protein, characterized in that, when a G protein-coupled receptor protein-activating compound, e.g. a ligand polypeptide of the present invention, etc. is contacted with G protein-coupled receptor proteins expressed on cell membranes by culturing transformants carrying G protein-coupled receptor protein-encoding DNA and when a G protein-coupled receptor protein-activating compound and a test compound are contacted with the G protein-coupled receptor protein expressed on the cell membrane by culturing the transformant carrying the G protein-coupled receptor protein-encoding DNA, the resulting G protein-coupled receptor protein-mediated cell stimulating activities, e.g. activities of promoting or activities of inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of

pH, activation of G protein, and cell promulgation, etc.; are measured and compared.

The G protein-coupled receptor agonist or antagonist have to be screened by, first, obtaining a candidate compound by using G protein-coupled receptor protein-containing cells, tissues or cell membrane fractions derived from rat or the like (primary screening), then, making sure whether the candidate compound really inhibitis the binding between human G protein-coupled receptor proteins and ligands (secondary screening). Other receptor proteins inevitably exist and when the cells, the tissues or the cell membrane fractions were used, they intrinsically make it difficult to screen agonists or antagonists to the desired receptor proteins. By using the human-derived G protein-coupled receptor protein, however, there is no need of effecting the primary screening, whereby it is possible to efficiently screen a compound that changes the binding activity between a ligand and a G protein-coupled receptor. Additionally, it is possible to evaluate whether the compound that is screened is a G protein-coupled receptor agonist or a G protein-coupled receptor antagonist.

Specific explanations of the screening method will be given as hereunder.

First, with respect to the G protein-coupled receptor protein used for the screening method of the present invention, any product may be used so far as it contains G protein-coupled receptor proteins or partial peptides thereof although the use of a membrane fraction of mammalian organs is preferable. However, human organs can be extremely scarce and, accordingly, G protein-coupled receptor proteins which are expressed in a large amount using a recombinant technique are suitable for the screening.

In the manufacture of the G protein-coupled

receptor protein, the above-mentioned method can be used.

When the G protein-coupled receptor protein-containing cells or cell membrane fractions are used in the screening method, the above-mentioned method can be used.

In conducting the above-mentioned methods 1) to 3) for screening the compound capable of changing the binding activity of the ligand with the G protein-coupled receptor protein, a suitable G protein-coupled receptor fraction and a labeled ligand polypeptide are necessary. With respect to the G protein-coupled receptor fraction, it is preferred to use naturally occurring G protein-coupled receptors (natural type G protein-coupled receptors) or recombinant type G protein-coupled receptor fractions with the activity equivalent to that of the natural type G protein coupled. Here the term "activity equivalent to" means the same ligand binding activity, or the substantially equivalent ligand binding activity.

With respect to the labeled ligand, it is possible to use labeled ligands, labeled ligand amalogized compounds, etc. For example, ligands labeled with [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc. and other labeled substances may be utilized.

Specifically, G protein-coupled receptor protein-containing cells or cell membrane fractions are first suspended in a buffer which is suitable for the determining method to prepare the receptor sample in conducting the screening for a compound which changes the binding activity of the ligand with the G protein-coupled receptor protein. With respect to the buffer, any buffer such as Tris-HCl buffer or phosphate buffer of pH 4-10, preferably, pH 6-8 which does not inhibit the binding of the ligand with the receptor may be used.

In addition, a surface-active agent such as CHAPS, Tween 80™ (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and/or various proteins such as bovine serum albumin (BSA), gelatine, etc. may be added to the buffer with an object of decreasing the nonspecific binding. Further, a protease inhibitor such as PMSF, leupeptin, E-64 manufactured by Peptide Laboratory, Japan, pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A labeled ligand in a certain amount (5,000 cpm to 500,000 cpm) is added to 0.01 ml to 10 ml of said receptor solution and, at the same time, 10^{-4}M to 10^{-10}M of a test compound coexists. In order to determine the nonspecific binding amount (NSB), a reaction tube to which a great excessive amount of unlabeled test compounds is added is prepared as well.

The reaction is carried out at 0-50°C, preferably at 4-37°C for 20 minutes to 24 hours, preferably 30 minutes to three hours. After the reaction, it is filtered through a glass fiber filter, a filter paper, or the like, washed with a suitable amount of the same buffer and the radioactivity retained in the glass fiber filter, etc. is measured by means of a liquid scintillation counter of a gamma-counter. Supposing that the count (B_0 - NSB) obtained by subtracting the nonspecific binding amount (NSB) from the total binding amount (B_0) wherein an antagonizing substance is not present is set at 100%, a test compound in which the specific binding amount (B - NSB) obtained by subtracting the nonspecific binding amount (NSB) from the total binding amount (B) is, for example, less than 50% may be selected as a candidate ligand to the G protein-coupled receptor protein of the present invention.

In conducting the above-mentioned methods 4) to 5)

for screening the compound which changes the binding activity of the ligand with the G protein-coupled receptor protein, the G protein-coupled receptor protein-mediated cell stimulating activity, e.g. activities of promoting or activities of inhibiting physiological responses such as release of arachidonic acid, release of acetylcholine, intracellular Ca^{2+} increase, intracellular cAMP production, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of intracullular proteins, activation of c-fos, lowering of pH, activation of G protein and cell proliferation, etc.; may be measured by known methods or by the use of commercially available measuring kits. To be more specific, G protein-coupled receptor protein-containing cells are at first cultured in a multiwell plate or the like.

In conducting the screening, it is substituted with a suitable buffer which does not show toxicity to fresh media or cells in advance, incubated under appropriate conditions and for a specified time after additing a test compound, etc. thereto. The resultant cells are extracted or the supernatant liquid is recovered and the resulting product is determined, preferably quantitatively, by each of the methods. When it is difficult to identify the production of the indicative substance, e.g. arachidonic acid, etc. which is to be an indication for the cell stimulating activity due to the presence of decomposing enzymes contained in the cell, an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activities such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the cAMP production in the cells whose fundamental production has been increased by forskolin or the like.

In conducting a screening by measuring the cell

stimulating activity, cells in which a suitable G protein-coupled receptor protein is expressed are necessary. Preferred G protein-coupled receptor protein-expressing cells are naturally occurring G protein-coupled receptor protein (natural type G protein-coupled receptor protein)-containing cell lines or strains, e.g. mouse pancreatic β cell line, MIN6, etc., the above-mentioned recombinant type G protein-coupled receptor protein-expressing cell lines or strains, etc.

Examples of the test compound includes peptide, proteins, non-peptidic compounds, synthesized compounds, fermented products, cell extracts, plant extracts, animal tissue extracts, serum, blood, body fluid, etc. Those compounds may be novel or known.

A kit for screening the compound which changes the binding activity of the ligand with the G protein-coupled receptor protein or a salt thereof comprises a G protein-coupled receptor protein or a partial peptide thereof, or G protein-coupled receptor protein-containing cells or cell membrane fraction thereof.

Examples of the screening kit include as follows:

1. Reagent for Determining Ligand.

1) Buffer for Measurement and Buffer for Washing.

The product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This may be sterilized by filtration through a membrane filter with a 0.45 μm pore size, and stored at 4°C or may be prepared upon use.

2) Sample of G protein-coupled receptor Protein.

CHO cells in which a G protein-coupled receptor protein is expressed are subcultured at the rate of 5×10^5 cells/well in a 12-well plate and cultured at 37°C with a 5% CO_2 and 95% air atmosphere for two days to prepare the sample.

3) Labeled Ligand.

The ligand which is labeled with commercially available [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc.

The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1 μM with a buffer for the measurement.

4) Standard Ligand Solution.

Ligand is dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make 1 mM and stored at -20°C.

2. Method of the Measurement.

1) CHO cells are cultured in a 12-well tissue culture plate to express G protein-coupled receptor proteins. The G protein-coupled receptor protein-expressing CHO cells are washed with 1 ml of buffer for the measurement twice. Then 490 μl of buffer for the measurement is added to each well.

2) Five μl of a test compound solution of 10^{-3} to 10^{-10} M is added, then 5 μl of a labeled ligand is added and is made to react at room temperature for one hour. For knowing the non-specific binding amount, 5 μl of the ligand of 10^{-3} M is added instead of the test compound.

3) The reaction solution is removed from the well, which is washed with 1 ml of buffer for the measurement three times. The labeled ligand binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (such as manufactured by Wako Pure Chemical, Japan).

4) Radioactivity is measured using a liquid scintillation counter (e.g., one manufactured by Beckmann) and PMB (percent maximum binding) is calculated by the following equation:

$$PMB = [(B - NSB)/(B_0 - NSB)] \times 100$$

PMB: Percent maximum binding

B: Value when a sample is added

NSB: Nonspecific binding

B_0: Maximum binding

The compound or a salt thereof obtained by the screening method or by the screening kit is a compound which changes the binding activity of a ligand polypeptide with a G protein-coupled receptor protein, wherein the compound inhibits or promotes the bonding, and, more particularly, it is a compound having a cell stimulating activity mediated via a G protein-coupled receptor or a salt thereof, so-called "G protein-coupled receptor agonist" or a compound having no said stimulating activity, so-called "G protein-coupled receptor antagonist". Examples of said compound are peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, etc. and the compound may be novel or known.

Said G protein coupled eceptor agonist has the same physiological action as the ligand to the G protein-coupled receptor protein has and, therefore, it is useful as a safe and less toxic pharmaceutical composition depending upon said ligand activity.

On the other hand, said G protein-coupled receptor antagonist is capable of inhibiting the physiological activity of the ligand to the G protein-coupled receptor protein and, therefore, it is useful as a safe and less toxic pharmaceutical composition for inhibiting said ligand activity.

The ligand polypeptide of the present invention relates to the pituitary function modulating action, central nervous system function modulating action or pancreatic function modulating action. Therefore, the above-mentioned agonist or antagonist can be used as a therapeutic and/or prophylactic agent for dementia such as senile dementia, cerebrovascular dementia (dementia due to cerebrovascular disorder), dementia associated

with phylodegenerative retroplastic diseases (e.g. Alzheimer's disease, Parkinson's disease, Pick's disease, Huntington's disease, etc.), dementia due to infectious diseases (e.g. delayed viral infections such as Creutzfelt-Jakob disease), dementia associated with endocrine, metabolic, and toxic diseases (e.g. hypothyroidism, vitamin B12 deficiency, alcoholism, and poisoning due to various drugs, metals, or organic compounds), dementia associated with oncogenous diseases (e.g. brain tumor), dementia due to traumatic diseases (e.g. chronic subdural hematoma):, depression (melancholia), hyperkinetic (microencephalo-pathy) syndrome, disturbance of consciousness, anxiety syndrome, schizophrenia, horror, growth hormone secretory disease (e.g. gigantism, acromegalic gigantism etc.), hyperphagia, polyphagia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, hyperprolactinemia, hypoglycemia, pituitarism, pituitary drawfism, diabetes (e.g. diabetic complications, diabetic nephropathy, diabetic neurophathy, diabetic retinopathy etc.), cancer (e.g. mammary cancer, lymphatic leukemia, cystic cancer, ovary cancer, prostatic cancer etc.), pancreatitis, renal disease (e.g. chromic renal failure, nephritis etc.), Turner's syndrome, neurosis, rheumatoid arthritis, spinal injury, transient brain ischemia, amyotrophic lateral sclerosis, acute myocardial infarction, spinocerebellar degeneration, bone fracture, trauma, atopic dermatitis, osteoporosis, asthma, epilepsy, infertility or oligogalactia. Furthermore, the agonist or antagonist can be also used as hypnotic-sedative, agent for improvement in postoperative nutritional status, vasopressor or depressor.

When the compound or the salt thereof obtained by the screening method or by the screening kit is used as

the pharmaceutical composition, a conventional means which is the same means as using above-mentioned ligand polypeptide as the pharmaceutical compoisiton may be applied therefor.

(5) Manufacture of Antibody or Antiserum against the Ligand Polypeptide or the G protein-coupled receptor Protein.

Antibodies, e.g. polyclonal antibody, monoclonal antibody, and antisera against the ligand polypeptide or the G protein-coupled receptor protein may be manufactured by antibody- or antiserum-manufacturing methods per se known to those of skill in the art or methods similar thereto, using the ligand polypeptide or the G protein-coupled receptor protein as antigen. For example, polyclonal antibodies can be manufactured by the method as given below.

[Preparation of a polyclonal antibody]

The above-mentioned polypeptide or protein as the antigen is coupled to a carrier protein. The carrier protein may for example be bovine thyroglobulin, bovine serum albumin, bovine gamma-globulin, hemocyanine, or Freund's complete adjuvant (Difco).

The coupling reaction between the antigen protein and the carrier protein can be carried out by the known procedure. The reagent for use in the coupling reaction includes but is not limited to glutaraldehyde and water-soluble carbodiimide. The suitable ratio of the antigen protein to the carrier protein is about 1:1 through about 1:10 and as to the reaction pH, satisfactory results are obtained in many cases when the reaction is carried out around neutral, particularly in the range of pH about 6-8. The reaction time is preferably about 1 to 12 hours in many cases and more preferably about 2 to 6 hours. The conjugate thus obtained is dialyzed against water at

about 0 to 18°C in the routine manner and stored frozen or optionally lyophilized and stored.

For the production of a polyclonal antibody, a warm-blooded animal is inoculated with the immunogen produced in the manner described hereinbefore. The warm-blooded animal that can be used for this purpose includes mammalian warm-blooded animals, e.g. rabbit, sheep, goat, rat, mouse, guinea pig, bovine, equine, swine, etc.; and avian species, e.g. chicken, dove, duck, goose, quail, etc. Regarding the methodology for inoculating a warm-blooded animal with the immunogen, the inoculum size of the immunogen may be just sufficient for antibody production. For example, the desired antibody can be produced in many instances by emulsifying 1 mg of the immunogen in 1 ml of saline with Freund's complete adjuvant and injecting the emulsion subcutaneously at the back and hind-limb footpad of rabbits 5 times at 4-week intervals. For harvesting the antibody produced in the warm-blooded animal, for example a rabbit, the blood is withdrawn from the auricular vein usually during day 7 through day 12 after the last inoculation dose and centrifuged to recover an antiserum. For purification, the antiserum is generally subjected to affinity chromatography using a carrier to which each antigen peptide has been conjugated and the adsorbed fraction is recovered to provide a polyclonal antibody.

The monoclonal antibody can be produced by the following method.

[Preparation of Monoclonal Antibody]

(a) Preparation of Monoclonal Antibody-Producing Cells.

The ligand polypeptide or G protein-coupled receptor protein is aministered to warm-blooded animals either solely or together with carriers or diluents to

the site where the production of antibody is possible by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and two to ten times in total. Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and chickens and the use of mice and rats is preferred.

In the preparation of the cells which produce monoclonal antibodies, an animal wherein the antibody titer is noted is selected from warm-blooded animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells to give monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by reacting a labeled ligand polypeptide or a labeled G protein-coupled receptor protein (which will be mentioned later) with the antiserum followed by measuring the binding activity of the labeling agent with the antibody. The operation for fusing may be carried out, for example, by a method of Koehler and Milstein (Nature, 256, 495, 1975), Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

Examples of the myeloma cells are NS-1, P3U1, SP2/0, AP-1, etc. and the use of P3U1 is preferred. The preferred fusion ratio of the numbers of antibody-producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10-80% followed by incubating at 20-40°C (preferably, at 30-37°C) for one to ten

minutes, an efficient cell fusion can be carried out.

Various methods may be applied for screening a hybridoma which produces anti-ligand polypeptide antibody or anti-G protein-coupled receptor antibody. For example, a supernatant liquid of hybridoma culture is added to a solid phase (e.g. microplate) to which the ligand polypeptide antigen or the G protein-coupled receptor protein antigen is adsorbed either directly or with a carrier, then anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody is used when the cells used for the cell fusion are those of mouse) which is labeled with a radioactive substance, an enzyme or the like, or protein A is added thereto and then anti-ligand polypeptide monoclonal antibodies or anti-G protein-coupled receptor monoclonal antibodies bound on the solid phase are detected; or a supernatant liquid of the hybridoma culture is added to the solid phase to which anti-immunoglobulin or protein A is adsorbed, then the ligand polypeptide or the G protein-coupled receptor labeled with a radioactive substance or an enzyme is added and anti-ligand polypeptide or anti-G protein-coupled receptor monoclonal antibodies bonded with the solid phase is detected.

Selection and cloning of the anti-ligand polypeptide monoclonal antibody- or the anti-G protein-coupled receptor monoclonal antibody-producing hybridoma may be carried out by methods <u>per se</u> known to those of skill in the art or methods similar thereto. Usually, it is carried out in a medium for animal cells, containing HAT (hypoxanthine, aminopterin and thymidine). With respect to a medium for the selection, for the cloning and for the growth, any medium may be used so far as hybridoma is able to grow therein. Examples of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical Co., Ltd., Japan) containing 1-20% (preferably 10-20%) of fetal calf

serum (FCS), a GIT medium (Wako Pure Chemical, Japan) containing 1-20% of fetal calf serum and a serum-free medium for hybridoma culturing (SFM-101; Nissui Seiyaku, Japan). The culturing temperature is usually 20-40°C and, preferably, about 37°C. The culturing time is usually from five days to three weeks and, preferably, one to two weeks. The culturing is usually carried out in 5% carbon dioxide gas. The antibody titer of the supernatant liquid of the hybridoma culture may be measured by the same manner as in the above-mentioned measurement of the antibody titer of the anti-ligand polypeptide or the anti-G protein-coupled receptor in the antiserum.

(b) Purification of the Monoclonal Antibody.

Like in the separation/purification of conventional polyclonal antibodies, the separation/purification of the anti-ligand polypeptide monoclonal antibody or the anti-G protein-coupled receptor monoclonal antibody may be carried out by methods for separating/purifying immunoglobulin such as salting-out, precipitation with an alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying methods in which only an antibody is collected by treatment with an active adsorbent such as an antigen-binding solid phase, protein A or protein G and the bond is dissociated whereupon the antibody is obtained.

The ligand polypeptide antibody or the G protein-coupled receptor antibody which is manufactured by the aforementioned method (a) or (b) is capable of specifically recognizing ligand polypeptide or G protein-coupled receptors and, accordingly, it can be used for a quantitative determination of the ligand polypeptide or the G protein-coupled receptor in test liquid samples and particularly for a quantitative

determination by sandwich immunoassays.

Thus, the present invention provides, for example, the following methods:

(i) a quantitative determination of a ligand polypeptide or a G protein-coupled receptor in a test liquid sample, which comprises

(a) competitively reacting the test liquid sample and a labeled ligand polypeptide or a labeled G protein-coupled receptor with an antibody which reacts with the ligand polypeptide or the G protein-coupled receptor, and

(b) measuring the ratio of the labeled ligand polypeptide or the labeled G protein-coupled receptor binding with said antibody; and

(ii) a quantitative determination of a ligand polypeptide or a G protein-coupled receptor in a test liquid sample, which comprises

(a) reacting the test liquid sample with an antibody immobilized on an insoluble carrier and a labeled antibody simultaneously or continuously, and

(b) measuring the activity of the labeling agent on the insoluble carrier

wherein one antibody is capable of recognizing the N-terminal region of the ligand polypeptide or the G protein-coupled receptor while another antibody is capable of recognizing the C-terminal region of the ligand polypeptide or the G protein-coupled receptor.

When the monoclonal antibody of the present invention recognizing a ligand polypeptide or G protein-coupled receptor (hereinafter, may be reffered to as "anti-ligand polypeptide or anti-G protein-coupled receptor antibody") is used, ligand polypeptide or G protein-coupled receptors can be measued and, moreover, can be detected by means of a tissue staining, etc. as well. For such an object, antibody molecules <u>per se</u> may be used or $F(ab')_2$, Fab' or Fab

fractions of the antibody molecule may be used too. There is no particular limitation for the measuring method using the antibody of the present invention and any measuring method may be used so far as it relates to a method in which the amount of antibody, antigen or antibody-antigen complex, depending on or corresponding to the amount of antigen, e.g. the amount of ligand polypeptide or G protein-coupled receptor, etc. in the liquid sample to be measured, is detected by a chemical or a physical means and then calculated using a standard curve prepared by a standard solution containing the known amount of antigen. For exmaple, nephrometry, competitive method, immunometric method and sanwich method are suitably used and, in terms of sensitivity and specificity, the sandwich method which will be described herein later is particularly preferred.

Examples of the labeling agent used in the measuring method using the labeling substance are radioisotopes, enzymes, fluorescent substances, luminescent substances, colloids, magnetic substances, etc. Examples of the radioisotope are [^{125}I], [^{131}I], [^{3}H] and [^{14}C]; preferred examples of the enzyme are those which are stable and with big specific activity, such as β-galactosidase, β-glucosidase, alkali phosphatase, peroxidase and malate dehydrogenase; examples of the fluorescent substance are fluorescamine, fluorescein isothiocyanate, etc.; and examples of the luminescent substance are luminol, luminol derivatives, luciferin, lucigenin, etc. Further, a biotin-avidin system may also be used for binding an antibody or antigen with a labeling agent.

In an insolubilization (immobilization) of antigens or antibodies, a physical adsorption may be used or a chemical binding which is usually used for insolubilization or immobilization of proteins or

enzymes may be used as well. Examples of the carrier are insoluble polysaccharides such as agarose, dextran and cellulose; synthetic resins such as polystyrene, polyacrylamide and silicone; glass; etc.

In a sandwich (or two-site) method, the test liquid is made to react with an insolubilized anti-ligand polypeptide or anti-G protein-coupled receptor antibody (the first reaction), then it is made to react with a labeled anti-ligand polypeptide or a labeled anti-G protein-coupled receptor antibody (the second reaction) and the activity of the labeling agent on the insoluble carrier is measued whereupon the amount of the ligand polypeptide or the G protein-coupled receptor in the test liquid can be determined. The first reaction and the second reaction may be conducted reversely or simultaneously or they may be conducted with an interval. The type of the labeling agent and the method of insolubilization (immobilization) may be the same as those mentioned already herein. In the immunoassay by means of a sandwich method, it is not always necessary that the antibody used for the labeled antibody and the antibody for the solid phase is one type or one species but, with an object of improving the measuring sensitivity, etc., a mixture of two or more antibodies may be used too.

In the method of measuring ligand polypeptide or G protein-coupled receptors by the sandwich method of the present invention, the preferred anti-ligand polypeptide antibodies or anti-G protein-coupled receptor antibodies used for the first and the second reactions are antibodies wherein their sites binding to the ligand polypeptide or the G protein-coupled receptors are different each other. Thus, the antibodies used in the first and the second reactions are those wherein, when the antibody used in the second reaction recognizes the C-terminal region of the ligand

polypeptide or the G protein-coupled receptor, then the antibody recognizing the site other than C-terminal regions, e.g. recognizing the N-terminal region, is preferably used in the first reaction.

The anti-ligand polypeptide antibody or the anti-G protein-coupled receptor antibody of the present invention may be used in a measuring system other than the sandwich method such as a competitive method, an immunometric method and a naphrometry. In a competitive method, an antigen in the test solution and a labeled antigen are made to react with an antibody in a competitive manner, then an unreacted labeled antigen (F) and a labeled antigen binding with an antibody (B) are separated (i.e. B/F separation) and the labeled amount of any of B and F is measured whereupon the amount of the antigen in the test solution is determined. With respect to a method for such a reaction, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F separation is conducted by polyethylene glycol, a second antibody to the above-mentioned antibody, etc.; and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an immobilized antibody is used as the second antibody.

In an immunometric method, an antigen in the test solution and an immobilized antigen are subjected to a competitive reaction with a certain amount of a labeled antibody followed by separating into solid and liquid phases; or the antigen in the test solution and an excess amount of labeled antibody are made to react, then a immobilized antigen is added to bind an unreacted labeled antibody with the solid phase and separated into solid and liquid phases. After that, the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

In a nephrometry, the amount of insoluble sediment which is produced as a result of the antigen-antibody reaction in a gel or in a solution is measured. Even when the antigen amount in the test solution is small and only a small amount of the sediment is obtained, a laser nephrometry wherein scattering of laser is utilized can be suitably used.

In applying each of those immunological measuring methods (immunoassays) to the measuring method of the present invention, it is not necessary to set up any special condition, operation, etc. therefor. A measuring system (assay system) for ligand polypeptide or G protein-coupled receptor may be constructed taking the technical consideration of the persons skilled in the art into consideration in the conventional conditions and operations for each of the methods. With details of those conventional technical means, a variety of reviews, reference books, etc. may be referred to. They are, for example, Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); Hiroshi Irie (ed): "Radioimmunoassay; Second Series" (Kodansha, Japan, 1979); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Igaku Shoin, Japan, 1978); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Second Edition) (Igaku Shoin, Japan, 1982); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Third Edition) (Igaku Shoin, Japan, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid. Vo. 73 (Immunochemical Techniques (Part B)); ibid. Vo. 74 (Immunochemical Techniques (Part C)); ibid. Vo. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid. Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid. Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (Academic Press); etc.

As such, the amount of ligand polypeptide or G protein-coupled receptor proteins can now be determined with a high precision using the anti-ligand polypeptide or the anti-G protein-coupled receptor antibody of the present invention.

In the specification and drawings of the present application, the abbreviations used for bases (nucleotides), amino acids and so forth are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature or those conventionally used in the art. Examples thereof are given below. Amino acids for which optical isomerism is possible are, unless otherwise specified, in the L form.

DNA : Deoxyribonucleic acid
cDNA : Complementary deoxyribonucleic acid
A : Adenine
T : Thymine
G : Guanine
C : Cytosine
RNA : Ribonucleic acid
mRNA : Messenger ribonucleic acid
dATP : Deoxyadenosine triphosphate
dTTP : Deoxythymidine triphosphate
dGTP : Deoxyguanosine triphosphate
dCTP : Deoxycytidine triphosphate
ATP : Adenosine triphosphate
EDTA : Ethylenediamine tetraacetic acid
SDS : Sodium dodecyl sulfate
EIA : Enzyme Immunoassay
G, Gly: Glycine (or Glycyl)
A, Ala: Alanine (or Alanyl)
V, Val: Valine (or Valyl)
L, Leu: Leucine (or Leucyl)
I, Ile: Isoleucine (or Isoleucyl)
S, Ser: Serine (or Seryl)
T, Thr: Threonine (or Threonyl)

C, Cys: Cysteine (or Cysteinyl)
M, Met: Methionine (or Methionyl)
E, Glu: Glutamic acid (or Glutamyl)
D, Asp: Aspartic acid (or Aspartyl)
K, Lys: Lysine (or Lysyl)
R, Arg: Arginine (or Arginyl)
H, His: Histidine (or Histidyl)
F, Phe: Phenylalamine (or Phenylalanyl)
Y, Tyr: Tyrossine (or Tyrosyl)
W, Trp: Tryptophan (or Tryptophanyl)
P, Pro: Proline (or Prolyl)
N, Asn: Asparagine (or Asparaginyl)
Q, Gln: Glutamine (or Glutaminyl)
pGlu: Pyroglutamic acid (or Pyroglutamyl)
Me: Methyl
Et: Ethyl
Bu: Butyl
Ph: Phenyl
TC: Thiazolidinyl-4(R)-carboxamide

In this specification, substitutions, protective groups and reagents commonly used are indicated by the following abbreviations:

BHA : benzhydrylamine
pMBHA : p-methylbenzhydrylamine
Tos : p-toluenesulfonyl
CHO : formyl
HONB : N-hydroxy-5-norbornene-2,3-dicarboxyimide
OcHex : cyclohexyl ester
Bzl : benzyl
Bom : benzyloxymethyl
Br-Z : 2-bromobenzyloxycarbonyl
Boc : t-butoxycarbonyl
DCM : dichloromethane
HOBt : 1-hydroxybenztriazole
DCC : N,N'-dicyclohexylcarbodiimide
TFA : trifluoro acetate

DIEA : diisopropylethylamine
Fmoc : N-9-fluorenylmethoxycarbonyl
DNP : dinitrophenyl
Bum : t-butoxymethyl
Trt : trityl

Each SEQ ID NO set forth in the SEQUENCE LISTING of the specification refers to the following sequence:

[SEQ ID NO:1] is an entire amino acid sequence of the bovine pituitary-derived ligand polypeptide encoded by the cDNA included in pBOV3.

[SEQ ID NO:2] is an entire nucleotide sequence of the bovine pituitary-derived ligand polypeptide cDNA.

[SEQ ID NO:3] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide which was obtained by purification and analysis of N-terminal sequence for P-3 fraction. The amino acid sequence corresponds to 23rd to 51st positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:4] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide which was obtained by purification and analysis of N-terminal sequence for P-2 fraction. The amino acid sequence corresponds to 34th to 52nd positions of the amino acid sequencce of SEQ ID NO:1.

[SEQ ID NO:5] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 23rd to 53rd positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:6] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 23rd to 54th positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:7] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 23rd to 55th positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:8] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 34th to 53rd positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:9] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 34th to 54th positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:10] is an amino acid sequence of the bovine pituitary-derived ligand polypeptide. The amino acid sequence corresponds to 34th to 55th positions of the amino acid sequence of SEQ ID NO:1.

[SEQ ID NO:11] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:3).

[SEQ ID NO:12] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:4).

[SEQ ID NO:13] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:5).

[SEQ ID NO:14] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:6).

[SEQ ID NO:15] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:7).

[SEQ ID NO:16] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:8).

[SEQ ID NO:17] is a nucleotide sequence of DNA coding for the bovine pituitary derived ligand polypeptide (SEQ ID NO:9).

[SEQ ID NO:18] is a nucleotide sequence of DNA coding for the bovine pituitary-derived ligand polypeptide (SEQ ID NO:10).

[SEQ ID NO:19] is a partial amino acid sequence of the human pituitary-derived G protein-coupled receptor protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment included in p19P2.

[SEQ ID NO:20] is a partial amino acid sequence of the human pituitary-derived G protein-coupled receptor protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA fragment include in p19P2.

[SEQ ID NO:21] is an entire amino acid sequence of the human pituitary-derived G protein-coupled receptor protein encoded by the human pituitary-derived G protein-coupled receptor protein cDNA include in phGR3.

[SEQ ID NO:22] is a partial amino acid sequence of the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein encoded by the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein cDNA fragment having a nucleotide sequence (SEQ ID NO:27), derived based upon the nucleotide sequences of the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein cDNA fragments each included in pG3-2 and pG1-10.

[SEQ ID NO:23] is a partial amino acid sequence of the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein encoded by p5S38.

[SEQ ID NO:24] is a nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA fragment include in p19P2.

[SEQ ID NO:25] is a nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNA fragment include in p19P2.

[SEQ ID NO:26] is an entire nucleotide sequence of the human pituitary-derived G protein-coupled receptor protein cDNa include in phGR3.

[SEQ ID NO:27] is a nucleotide sequence of the mouse

pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein cDNA, derived based upon the nucleotide sequences of the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein cDNA fragments each included in pG3-2 and pG1-10.

[SEQ ID NO: 28] is a nucleotide sequence of the mouse pancreatic β-cell line, MIN6-derived G protein-coupled receptor protein cDNA include in p5S38.

[SEQ ID NO:29] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:30] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:31] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:32] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:33] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:34] is a synthetic DNA primer for screening of cDNA coding for the G protein-coupled receptor protein.

[SEQ ID NO:35] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by P5-1.

[SEQ ID NO:36] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by P3-1.

[SEQ ID NO:37] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by P3-2.

[SEQ ID NO:38] is a synthetic DNA primer for screening

of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by PE.

[SEQ ID NO:39] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by PDN.

[SEQ ID NO:40] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by FB.

[SEQ ID NO:41] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by FC.

[SEQ ID NO:42] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by BOVF.

[SEQ ID NO:43] is a synthetic DNA primer for screening of cDNA coding for the bovine pituitary-derived ligand polypeptide, wherein the primer is represented by BOVR.

[SEQ ID NO:44] is an entire amino acid sequence of the bovine genome-derived ligand polypeptide.

[SEQ ID NO: 45] is an entire amino acid sequence of the rat type ligand polypeptide encoded by the cDNA included in pRAV3.

[SEQ ID NO:46] is an entire nucleotide sequence of the rat type ligand polypeptide cDNA.

[SEQ ID NO:47] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 22nd to 52nd positions of the amino acid sequence of SEQ ID NO:45.

[SEQ ID NO:48] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 22nd to 53rd positions of the amino acid sequence of SEQ ID NO:45.

[SEQ ID NO:49] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 22nd to 54th positions of the amino acid sequence of SEQ ID NO:45.

[SEQ ID NO:50] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 33rd to 52nd positions of the amino acid sequence of SEQ ID NO:45.

[SEQ ID NO:51] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 33rd to 53rd positions of the amino acid sequence of SEQ ID NO:45.

[SEQ ID NO:52] is an amino acid sequence of the rat type ligand polypeptide. The amino acid sequence corresponds to 33rd to 54th positions of the amino acid sequence of SEQ ID NO.45.

[SEQ ID NO:53] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:47.

[SEQ ID NO:54] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:48.

[SEQ ID NO:55] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:49.

[SEQ ID NO:56] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:50.

[SEQ ID NO:57] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:51.

[SEQ ID NO:58] is a nucleotide sequence encoding for the rat type ligand polypeptide of SEQ ID NO:52.

[SEQ ID NO:59] is an entire amino acid sequence of the human type ligand polypeptide encoded by the cDNA includedin pHOB7.

[SEQ ID NO:60] is an entire nucleotide sequence of the human type ligand polypeptide cDNA.

[SEQ ID NO:61] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 23rd to 53rd positions of the amino acid sequence of SEQ ID NO.59.

[SEQ ID NO:62] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 23rd to 54th positions of the amino acid

sequence of SEQ ID NO.59.

[SEQ ID NO:63] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 23rd to 55th positions of the amino acid sequence of SEQ ID NO.59.

[SEQ ID NO:64] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 34th to 53rd positions of the amino acid sequence of SEQ ID NO.59.

[SEQ ID NO:65] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 34th to 54th positions of the amino acid sequence of SEQ ID NO.59.

[SEQ ID NO:66] is an amino acid sequence of the human type ligand polypeptide. The amino acid sequence corresponds to 34th to 55th positions of the amino acid sequence of SEQ ID NO.59.

[SEQ ID NO:67] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:61.

[SEQ ID NO:68] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:62.

[SEQ ID NO:69] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:63.

[SEQ ID NO:70] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:64.

[SEQ ID NO:71] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:65.

[SEQ ID NO:72] is a nucleotide sequence encoding for the human type ligand polypeptide of SEQ ID NO:66.

[SEQ ID NO:73] is a partial amino acid sequence of the ligand polypeptide, wherein Xaa of the 10th position is Ala or Thr, Xaa of the 11th position is Gly or Ser and Xaa of the 21st position is H, Gly or GlyArg.

[SEQ ID NO:74] is a partial amino acid sequence of the ligand polypeptide, wherein Xaa of the 3rd position is Ala or Thr, Xaa of the 5th position is Gln or Arg and

Xaa of the 10th position is Ile or Thr.

[SEQ ID NO:75] is a synthetic DNA primer for screening of cDNA coding for the rat type ligand polypeptide, wherein the primer is represented by RA.

[SEQ ID NO:76] is a synthetic DNA primer for screening of cDNA coding for the rat type ligand polypeptide, wherein the primer is represented by RC.

[SEQ ID NO:77] is a synthetic DNA primer for screening of cDNA coding for the rat type ligand polypeptide, wherein the primer is represented by rF.

[SEQ ID NO:78] is a synthetic DNA primer for screening of cDNA coding for the rat type ligand polypeptide, wherein the primer is represented by rR.

[SEQ ID NO:79] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by R1.

[SEQ ID NO:80] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by R3.

[SEQ ID NO:81] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by R4.

[SEQ ID NO:82] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by HA.

[SEQ ID NO:83] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by HB.

[SEQ ID NO:84] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by HE.

[SEQ ID NO:85] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by HF.

[SEQ ID NO:86] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide,

wherein the primer is represented by 5H.

[SEQ ID NO:87] is a synthetic DNA primer for screening of cDNA coding for the human type ligand polypeptide, wherein the primer is represented by 3HN.

[SEQ ID NO:88] is a synthetic DNA primer for screening of cDNA coding for the rat type G protein-coupled receptor protein (UHR-1), wherein the primer is represented by rRECF.

[SEQ ID NO:89] is a synthetic DNA primer for screening of cDNA coding for the rat type G protein-coupled receptor protein (UHR-1), wherein the primer is represented by rRECR.

[SEQ ID NO:90] is a synthetic DNA which is used for amplification of G3PDH, UHR-1 and ligand, wherein the primer represented by r19F.

[SEQ ID NO:91] is a synthetic DNA which is used for amplification of G3PDH, UHR-1 and ligand, wherein the primer represented by r19R.

[SEQ ID NO:92] is a N-terminal peptide of the ligand polypeptide, which is used for antigen. (Peptide-I)

[SEQ ID NO:93] is a C-terminal peptide of the ligand polypeptide, which is used for antigen. (Peptide-II)

[SEQ ID NO:94] is a peptide of the central portion in ligand polypeptide, which is used for antigen. (Peptide-III)

[SEQ ID NO:95] is a synthetic DNA primer for screening of cDNA coding for rat type G protein-coupled receptor protein (UHR-1).

[SEQ ID NO:96] is a synthetic DNA primer for screening of cDNA coding for rat type G protein-coupled receptor protein (UHR-1).

The transformant Escherichia coli, designated INVαF'/p19P2, which is obtained in the Example 2 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with the National Institute of Bioscience and Human-Technology

(NIBH), Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan and has been assigned the Accession Number FERM BP-4776. It is also on deposit from August 22, 1994 with the Institute for Fermentation, Osaka, Japan (IFO) and has been assigned the Accession Number IFO 15739.

The transformant Escherichia coli, designated INVαF'/pG3-2, which is obtained in the Example 4 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned the Accession Number FERM BP-4775. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15740.

The transformant Escherichia coli, designated JM109/phGR3, which is obtained in the Example 5 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4807. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession Number IFO 15748.

The transformant Escherichia coli, designated JM109/p5S38, which is obtained in the Example 8 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4856. It is also on deposit from October 25, 1994 with IFO and has been assigned the Accession Number IFO 15754.

The transformant Escherichia coli, designated JM109/pBOV3, which is obtained in the Example 20 mentioned herein below, is on deposit under the terms of the Budapest Treaty from February 13, 1996, with NIBH and has been assigned the Accession Number FERM BP-5391. It is also on deposit from January 25, 1996

with IFO and has been assigned the Accession Number IFO 15910.

The transformant Escherichia coli, designated JM109/pRAV3, which is obtained in the Example 29 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 12, 1996, with NIBH and has been assigned the Accession Number FERM BP-5665. It is also on deposit from September 3, 1996 with IFO and has been assigned the Accession Number IFO 16012.

The transformant Escherichia coli, designated JM109/pHOV7, which is obtained in the Example 32 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 12, 1996, with NIBH and has been assigned the Accession Number FERM BP-5666. It is also on deposit from September 5, 1996 with IFO and has been assigned the Accession Number IFO 16013.

[Industrial Application]

The bioactive substance of the present invention, namely the ligand polypeptide or its amide or ester thereof, or a salt thereof, a partial peptide thereof, or the DNA coding for said ligand polypeptide, has function modulating activity for various tissues or internal organs, e.g. heart, lung, liver, spleen, thymus, kidney, adrenal glands, skeltal muscle, testis etc., besides pituitary, central nervous system or pancreas, and are useful as medicines. Furthermore, the substance is useful for the screening of agonists or antagonists of G protein-coupled receptor proteins. The compounds which can be obtained by such screening also have function modulating activity for above-described tissues or internal organs, and are useful as medicines.

[Examples]

Described below are working examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention.

[Reference Example 1]

Preparation of Synthetic DNA Primer for Amplifying DNA Coding for G protein-coupled receptor Protein

A compariton of deoxyribonucleotide sequences coding for the known amino acid sequences corresponding to or near the first membrane-spanning domain each of human-derived TRH receptor protein (HTRHR), human-derived RANTES receptor protein (L10918, HUMRANTES), human Burkitt's lymphoma-derived unknown ligand receptor protein (X68149, HSBLR1A), human-derived somatostatin receptor protein (L14856, HUMSOMAT), rat-derived μ-opioid receptor protein (U02083, RNU02083), rat-derived κ-opioid receptor protein (U00442, U00442), human-derived neuromedin B receptor protein (M73482, HUMNMBR), human-derived muscarinic acetylcholine receptor protein (X15266, HSHM4), rat-derived adrenaline α_1B receptor protein (L08609, RATAADRE01), human-derived somatostatin 3 receptor protein (M96738, HUMSSTR3X), human-derived C_5a receptor protein (HUMC5AAR), human-derived unknown ligand receptor protein (HUMRDC1A), human-derived unknown ligand receptor protein (M84605, HUMOPIODRE), and rat-derived adrenaline α_2B receptor protein (M91466, RATA2BAR) was made. As a result, highly homologous regions or parts were found.

Further, a comparison of deoxynucleotide sequences coding for the known amino acid sequences corresponding to or near the sixth membrane-spanning domain each of mouse-derived unknown ligand receptor protein (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2

receptor protein (S46950, S46950), mouse-derived unknown ligand receptor protein (D21061, MUSGPCR), mouse-derived TRH receptor protein (S43387, S43387), rat-derived neuromedin K receptor protein (J05189, RATNEURA), rat-derived adenosine A1 receptor protein (M69045, RATA1ARA), human-derived neurokinin A receptor protein (M57414, HUMNEKAR), rat-derived adenosine A3 receptor protein (M94152, DATADENREC), human-derived somatostatin 1 receptor protein (M81829, HUMSRI1A), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived unknown ligand receptor protein (X61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR4Z) and rat-derived GnRH receptor protein (M31670, RATGNRHA) was made. As a result, highly homologous regions or parts were found.

The aforementioned abbreviations in the parentheses are identifiers (reference numbers) which are indicated when GenBank/EMBL Data Bank is retrieved by using DNASIS Gene/Protein Sequencing Data Base (CD019, Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as disclosed in Japanese Patent Publication No. 304797/1993 (EPA 638645).

Specifically, it was planned to incorporate mixed bases relying upon the base regions that were in agreement with cDNAs coding for a large number of receptor proteins in order to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions. Based upon these sequences, the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO:29 or SEQ ID NO:30 which is complementary to the homologous nucleotide sequence were produced.

[Synthetic DNAs]

5'-CGTGG (G or C) C (A or C) T (G or C) (G or C) TGGGCAAC (A, G, C or T) (C or T) CCTG-3'

(SEQ ID NO:29)

5'-GT (A, G, C or T) G (A or T) (A or G) (A or G) GGCA (A, G, C or T) CCAGCAGA (G or T) GGCAAA-3'

(SEQ ID NO:30)

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide resides in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis.

[Example 1]

Amplification of Receptor cDNA by PCR Using Human Pituitary Gland-Derived cDNA

By using human pituitary gland-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Reference Example 1 was carried out. The composition of the reaction solution consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 1 μM, 1 ng of the template cDNA, 0.25 mM dNTPs, 1 μl of Taq DNA polymerase and a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μl. The cycle for amplification including 95°C for 1 min., 55°C for 1 min. and 72°C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95°C for 5 minutes and at 65°C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

[Example 2]

Subcloning of PCR Product into Plasmid Vector and

Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned into the plasmid vector, pCR™II (TM represents registered trademark). The recombinant vectors were introduced into E. coli INVαF' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INVαF'/p19P2.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the

determined nucleotide sequences [SEQ ID NO:24 and 25 (Here, the determined nucleotide sequence is the nucleotide sequence which the underlined portion is deleted from the sequence of Figure 1 or Figure 2 respectively)].

As a result, it was learned that a novel G protein-coupled receptor protein was encoded by the cDNA fragment insert in the plasmid, p19P2, possessed by the transformant Escherichia coli INVαF'/p19P2. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid sequences [SEQ ID NO:19 and 20], and homology retrieval was carried out in view of hydrophobicity plotting [Figures 3 and 4] and at the amino acid sequence level to find homology relative to neuropeptide Y receptor proteins [Figure 5].

[Example 3]

Preparation of Poly(A)$^+$RNA Fraction from Mouse Pancreatic β-Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β-cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979) and, then, poly(A)$^+$RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μg of the poly(A)$^+$RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with mouse Moloney Leukemia virus (MMLV) reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μl of TE buffer (10 mM Tris-HCL at pH8.0, 1 mM EDTA at pH8.0).

[Example 4]

Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By suing, as a template, 5 μl of cDNA prepared from the mouse pancreatic β-cell strain, MIN6 in the above Example 3, PCR amplification using the DNA primers synthesized in Reference Example 1 was carried out under the same condition as in Example 1. The resulting PCR product was subcloned into the plasmid vector, pCR™II, in the same manner as in Example 2 to obtain a plasmid, pG3-2. The plasmid pG3-2 was transfected into E. coli INVαF' to obtain transformed Escherichia coli INVαF'/pG3-2.

By using, as a template, 5 μl of the cDNA parepared from the mouse pancreatic β-cell strain, MIN6, PCR amplification using DNA primers as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a degenerate synthetic primer represented by the following sequence:

5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT (G or T) GA (C or T) (A or C) G (G or C) TAC-3'

(SEQ ID NO:31)

wherein I is inosine; and

a degenerate synthetic primer represented by the following sequence:

5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI (G or C) (A or G) (C or T) GAA-3'

(SEQ ID NO:32)

wherein I is inosine,

was carried out under the same conditions as in Working Example 1. The resulting PCR product was subcloned into the plasmid vector, pCR™II, in the same manner as described in Example 2 to obtain a plasmid, pG1-10.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was

decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 6 shows a mouse pancreatic β-cell strain MIN6-derived G protein-coupled receptor protein-encoding DNA (SEQ ID NO:27) and an amino acid sequence (SEQ ID NO:22) encoded by the isolated DNA based upon the nucleotide sequences of plasmids pG3-2 and pG1-10 which are held by the transformant Escherichia coli INVαF'/pG3-2. The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the determined necleotide sequence [Figure 6]. As a result, it was learned that a novel G protein-coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence was converted into an amino acid sequence [Figure 6], hydrophobicity plotting was carried out to confirm the presence of six hydrophobic regions [Figure 8]. Upon comparing the amino acid sequence with that of p19P2 obtained in Example 2, furthermore, a high degree of homology was found as shown in [Figure 7]. As a result, it is strongly suggested that the G protein-coupled receptor proteins encoded by pG3-2 and pG1-10 recognize the same ligand as the G protein-coupled receptor protein encoded by p19P2 does while the animal species from which the receptor proteins encoded by pG3-2 and pG1-10 are derived is different from that from which the receptor protein encoded by p19P2 is.

[Example 5]

Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human Pituitary Gland-Derived cDNA Library

The DNA library constructed by Clontech Co. wherein λ gt11 phage vector is used (CLONTECH Laboratories, Inc.; CLH L1139b) was employed as a human pituitary gland-derived cDNA library. The human pituitary gland cDNA library (2×10^6 pfu (plaque forming units)) was mixed with E. coli Y1090$^-$ treated with magnesium sulfate, and incubated at 37°C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB. The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 μg/ml of ampicillin). A nitrocellulose filter was placed on the plate on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and then heated at 80°C for 3 hours to fix DNAs.

The filter was incubated overnight at 42°C together with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaCl, 0.2 M $NaH_2PO_4 \cdot H_2O$, 25 mM EDTA), 5 X Denhardt's solution (Nippon Gene, Japan), 0.1% SDS and 100 μg/ml of salmon sperm DNA for hybridization.

The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p19P2, obtained in Working Example 2, with EcoRI, followed by recovery and labelling by incorporation of [^{32}P]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

It was washed with 2 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at 55°C for 1 hour and, then, subjected to an autoradiography at -80°C to detect hybridized plaques.

In this screening, hybridization signals were recognized in three independent plaques. Each DNA was prepared from the three clones. The DNAs digested with EcoRI were subjected to an agarose electrophoresis and were analyzed by the southern blotting using the same probe as the one used in the screening. Hybridizing

bands were identified at about 0.7kb, 0.8kb and 2.0kb, respectively. Among them, the DNA fragment corresponding to the band at about 2.0kb (λ hGR3) was selected. The λ hGR3-derived EcoRI fragment with a hybridizable size was subcloned to the EcoRI site of the plasmid, pUC18, and E. coli JM109 was transformed with the plasmid to obtain transformant E. coli JM109/phGR3. A restriction enzyme map of the plasmid, phGR3, was prepared relying upon a restriction enzyme map deduced from the nucleotide sequence as shown in Example 2. As a result, it was learned that it carried a full-length receptor protein-encoding DNA which was predicted from the receptor protein-encoding DNA as shown in Example 2.

[Example 6]

Sequencing of Human Pituitary Gland-Derived Receptor Protein cDNA

Among the EcoRI fragments inserted in the plasmid, phGR3, obtained in the above Example 5, the from EcoRI to NheI nucleotide sequence with about 1330bp that is considered to be a receptor protein-coding region was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the EcoRI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare template plasmids for analyzing the nucleotide sequence.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 9 shows a nucleotide sequence of from immediate after the EcoRI site up to the NheI site encoded by phGR3. The nucleotide sequence of the human

pituitary gland-derived receptor protein-encoding DNA corresponds to the nucleotide sequence (SEQ ID NO:26) of from 118th to 1227th nucleotides [Figure 9]. An amino acid sequence of the receptor protein that is encoded by the nucleotide sequence is shown in SEQ ID NO:21.

[Example 7]

Northern Hybridization with Human Pituitary Gland-Derived Receptor Protein-Encoding phGR3

Northern blotting was carried out in order to detect the expression of phGR3-encoded human pituitary gland-derived receptor proteins obtained in Example 5 in the pituitary gland at a mRNA level. Human pituitary gland mRNA (2.5 µg, Clontech Co.) was used as a template mRNA and the same as the probe used in Working Example 5 was used as a probe. Nylon membrane (Pall Biodyne, U.S.A.) was used as a filter for northern blotting and migration of the mRNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989.

The hybridization was effected by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 µg/ml of salmon sperm DNA overnight at 42°C. The filter was washed with 0.1 x SSC, 0.1% SDS at 50°C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for three days at -80°C. The results were as shown in Figure 10 from which it is considered that the receptor gene encoded by phGR3 is expressed in the human pituitary gland.

[Example 8]

Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 µl of cDNA prepared

from the mouse pancreatic β-cell strain, MIN6 in Working Example 3, PCR amplification using the DNA primers synthesized in Example 4 as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a synthetic primer represented by the following sequence:
5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT (G or T) GA (C or T) (A or C) G (G or C) TAC-3'
(SEQ ID NO:31)

wherein I is inosine; and
a synthetic primer represented by the following sequence:
5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI (G or C) (A or G) (C or T) GAA-3'
(SEQ ID NO:32)

wherein I is inosine, was carried out under the same conditions as in Example 1. The resulting PCR product was subcloned to the plasmid vector, pCR™II, in the same manner as in Example 2 to obtain a plasmid, p5S38. The plasmid p5S38 was transfected into E. coli JM109 to obtain transformant Escherichia coli JM109/p5S38.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were read with DNASIS (Hitachi System Engineering Co., Japan).

Figure 12 showns a mouse pancreatic β-cell strain MIN6-derived G protein-coupled receptor protein-encoding DNA (SEQ ID NO:28) and an amino acid sequence (SEW ID NO:23) encoded by the isolated DNA based upon the nucleotide sequence of plasmid, p5S38. The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 12]. As a

result, it was learned that a novel G protein-coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan), the nucleotide sequence was converted into an amino acid sequence [Figure 12], and hydrophobicity plotting was carried out to confirm the presence of four hydrophobic regions [Figure 14]. Upon comparing the amino acid sequence with those encoded by p19P2 obtained in Example 2 and encoded by pG3-2 obtained in Example 4, furthermore, a high degree of homology was found as shown in Figure 13. As a result, it is strongly suggested that the mouse pancreatic β-cell strain, MIN6-derived G protein-coupled receptor protein encoded by p5S38 recognizes the same ligand as the human pituitary gland-derived G protein-coupled receptor protein encoded by p19P2 does while the animal species from which the receptor protein encoded by p5S38 is derived is different from that from which the receptor protein encoded by p19P2 is. It is also strongly suggested that the mouse pancreatic β-cell strain, MIN6-derived G protein-coupled receptor protein encoded by p5S38 recognized the same ligand as the mouse pancreatic β-cell strain, MIN6-derived G protein-coupled receptor proteins encoded by pG3-2 and pG1-10 do and they are analogous receptor proteins one another (so-called "subtype").

[Example 9]

Preparation of CHO cells which express phGR3

The plasmid phGR3 (Example 5) containing a cDNA encoding the full-length amino acid sequence of human pituitary receptor protein was digested with the restriction enzyme Nco I and electrophoresed on agarose gel and a fragment of about 1kb was recovered. Both ends of the recovered fragment were blunted with a DNA blunting kit (Takara Shuzo Co., Japan) and, with the

SalI linker added, treated with SalI and inserted into the SalI site of pUC119 to provide plasmid S10. Then, S10 was treated with SalI and SacII to prepare a fragment of about 700 bp (containing the N-terminal coding region). Then, a fragment of about 700 bp (containing the C-terminal coding region including initiation and termination codons) was cut out from phGR3 with Sac II and Nhe I. These two fragments were added to the animal cell expression vector plasmid pAKKO-111H (the vector plasmid identical to the pAKKO1.11 H described in Biochim. Biophys. Acta, Hinuma, S., et al., 1219 251-259, 1994) and a ligation reaction was carried out to construct a full-length receptor protein expression plasmid pAKKO-19P2.

E. coli transfected with pAKKO-19P2 was cultured and the pAKKO-19P2 plasmid DNA was mass-produced using QUIAGEN Maxi. A 20 µg portion of the plasmid DNA was dissolved in 1 ml of sterile PBS, and in a gene transfer vial (Wako Pure Chemical Ind.), the solution was vortexed well for riposome formation. This riposome, 125 µl, was added to CHOdhfr$^-$ cells subcultured at 1 x 10^6 per 10cm-dia. dish 24 hr before and placed in fresh medium immediately before addition and overnight culture was carried out. After a further one-day culture in fresh medium, the medium was changed to a screening medium and the incubation was further carried out for a day. For efficient screening of transformants, subculture was carried out at a low cell density and only the cells growing in the screening medium were selected to establish a full-length receptor protein expression CHO cell line CHO-19P2.

[Example 10]

Confirmation of the amount of expression of the full-length receptor protein in the CHO-19P2 cell line at the transcription level

Using FastTrack Kit (Invitrogen), CHO cells

transfected with pAKKO-19P2 according to the kit manual and mock CHO cells were used to prepare poly(A)$^+$RNA. Using 0.02 μg of this poly(A)$^+$RNA, a cDNA was synthesized by means of RNA PCR Kit (Takara Shuzo, Co., Japan). The kind of primer used was a random 9mer and the total volume of the reaction mixture was 40 μl. As a negative control of cDNA synthesis, a reverse transcriptase-free reaction mixture was also provided. First, the reaction mixture was incubated at 30°C for 10 minutes to conduct an amplification reaction to some extent. Then, it was incubated at 42°C for 30 minutes to let the reverse transcription reaction proceed. The enzyme was inactivated by heating at 99°C for 5 minutes and the reaction system was cooled at 5°C for 5 minutes.

After completion of the reverse transcription reaction, a portion of the reaction mixture was recovered and after dilution with distilled water, extraction was carried out with phenol/chloroform and further with diethyl ether. The extract was subjected to precipitation from ethanol and the precipitate was dissolved in a predetermined amount of distilled water for use as a cDNA sample. This cDNA solution and the plasmid DNA (pAKKO-19P2) were serially diluted and using primers specific to full-length receptor protein, PCR was carried out. The sequences of the primers prepared according to the base sequence of the coding region of the full-length receptor protein were CTGACTTATTTTCTGGGCTGCCGC (SEQ ID NO:33) for 5′ end and AACACCGACACATAGACGGTGACC (SEQ ID NO:34) for 3′ end.

The PCR reaction was carried out in a total volume of 100 μl using 1 μM each of the primers, 0.5 μl of Taq DNA polymerase (Takara Shuzo Co., Japan), the reaction buffer and dNTPs accompanying the enzyme, and 10 μl of template DNA (cDNA or plasmid solution). First the reaction mixture was heat-treated at 94°C for 2 minutes

for sufficient denaturation of the template DNA and subjected to 25 cycles of 95°C x 30 seconds, 65°C x 30 seconds, and 72°C x 60 seconds. After completion of the reaction, 10 µl of the reaction mixture was subjected to agarose gel electrophoresis and the detection and quantitative comparison of amplification products were carried out. As a result, a PCR product of the size (400 bp) predictable from the sequence of the cDNA coding for the full-length receptor protein was detected [Fig. 15]. In the lane of the PCR reaction mixture using the product of the reverse transcriptase-free transcription system as the template, no specific band was detected, thus extruding the possibility of its being a PCR product derived from the genomic DNA of CHO cells. Moreover, no specific band appeared in the lane of mock cells, either. Therefore, it was clear that the product was not derived from the mRNA initially expressed in CHO cells [Fig. 15].

[Example 11]

Detection of the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in a rat whole brain extract

A crude peptide fraction was prepared from rat whole brain by the following procedure. The rat whole brain enucleated immediately after sacrifice was frozen in liquefied nitrogen and stored at -80°C. The frozen rat whole brain, 20 g (the equivalent of 10 rats) was finely divided and boiled in 80 ml of distilled water for 10 minutes. After the boiled tissue was quenched on ice, 4.7 ml of acetic acid was added at a final concentration of 1.0 M and the mixture was homogenized using a Polytron (20,000 rpm, 6 min.). The homogenate was stirred overnight and then centrifuged (10,000 rpm, 20 min.) to separate the supernatant. The sediment was homogenized in 40 ml of 1.0 M acetic acid and centrifuged again to recover the supernatant. The

supernatants were pooled, diluted in 3 volumes of acetone, allowed to stand on ice for 30 minutes, and centrifuged (10,000 rpm, 20 min.) to recover the supernatant. The recovered supernatant was evaporated to remove acetone. To the resulting acetone-free concentrate was added 2 volumes of 0.05% trifluoroacetic acid(TFA)/H_2O and the mixture was applied to a reversed-phase C18 column (Prep C18 125Å, Millipore). After application of the supernatant, the column was washed with 0.05% TFA/H_2O, and gradient elution was carried out with 10%, 20%, 30%, 40%, 50%, and 60% CH_3CN/0.05%TFA/H_2O. The fractions were respectively divided into 10 equal parts and lyophilized. The dried sample derived from one animal equivalent of rat whole brain was dissolved in 20 µl of dimethyl sulfoxide (DMSO) and suspended in 1 ml of Hank's balanced saline solution (HBSS) supplemented with 0.05% bovine serum albumin (BSA) to provide a crude peptide fraction.

The full-length receptor protein-expressed CHO cells and mock CHO cells were seeded in a 24-well plate, 0.5 x 10^5 cells/well, and cultured for 24 hours. Then, [^{3}H] arachidonic acid was added at a final concentration of 0.25µCi/well. Sixteen (16) hours after addition of [^{3}H] arachidonic acid, the cells were rinsed with 0.05% BSA-HBSS and the above-mentioned crude peptide fraction was added, 400 µl/well. The mixture was incubated at 37°C for 30 minutes and a 300 µl portion of the reaction mixture (400 µl) was added to 4 ml of a scintillator and the amount of [^{3}H] arachidonic acid metabolite released into the reaction mixture was determined with a scintillation counter. As a result, an arachidonic acid metabolite-releasing activity specific to the full-length receptor protein expressed CHO cells (CHO-19P2) was detected in the 30%

CH_3CN fraction of the eluate [Fig. 16].

[Example 12]

Detection of the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells in a bovine hypothalamus extract

A crude peptide fraction was prepared from 360 g (the equivalent of 1 animals) of bovine brain tissue including hypothalamus in the same manner as in Example 11. A dried peptide sample per 0.05 animal was dissolved in 40 µl of DMSO and suspended in 2 ml of 0.05% BSA-HBSS and the detection of arachidonic acid metabolite-releasing activity was attempted in the same manner as in Example 11. As a result, the activity to specifically promote release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in the fraction eluted with 30% CH_3CN from a C18 column to which the crude bovine hypothalamus peptide fraction had been applied [Fig. 17].

[Example 13]

Preparation of the activity (peptide) to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells by purification from bovine hypothalamus

A typical process for harvesting the activity to specifically promote release of arachidonic acid metabolites from the CHO-19P2 cell line by purification from bovine hypothalamus is now described. A frozen bovine brain tissue specimen including hypothalamus, 4.0 kg (the equivalent of 80 animals) was ground and boiled in 8.0 L of distilled water for 20 minutes. After quenching on ice, 540 ml of acetic acid was added at a final concentration of 1.0 M and the mixture was homogenized using a Polytron (10,000 rpm, 12 min.). The homogenate was stirred overnight and then centrifuged (9,500 rpm, 20 min) to recover a supernatant. The sediment was suspended in 4.0 L of

1.0 M acetic acid and homogenized with the Polytron and centrifuged again to recover a further supernatant. The supernatants were pooled and TFA was added at a final concentration of 0.05%. The mixture was applied to reversed-phase C18 (Prep C18 125Å, 160 ml; Millipore) packed in a glass column. After addition, the column was washed with 320 ml of 0.05% TFA/H_2O and 3-gradient elution was carried out with 10%, 30%, and 50% CH_3CN/0.05% TFA/H_2O. To the 30% CH_3CN/0.05% TFA/H_2O fraction was added 2 volumes of 20 mM CH_3COONH_4/H_2O and the mixture was applied to the cation exchange column HiPrep CM-Sepharose FF (Pharmacia). After the column was washed with 20 mM CH_3COONH_4/10% CH_3CN/H_2O, 4-gradient elution was carried out with 100 mM, 200 mM, 500 mM, and 1000 mM CH_3COONH_4/10% CH_3CN/H_2O. In the 200 mM CH_3COONH_4 fraction, activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 was detected. Therefore, this fraction was diluted with 3 volumes of acetone, centrifuged for deproteination, and concentrated in an evaporator. To the concentrated fraction was added TFA (final concentration 0.1%) and the mixture was adjusted to pH 4 with acetic acid and applied to 3 ml of the reversed-phase column RESOURCE RPC (Pharmacia). Elution was carried out on a concentration gradient of 15%-30% CH_3CN. As a result, activity to specifically promote the release of arachidonic acid metabolites from the CHO-19P2 cell line was detected in the 19%-21% CH_3CN fraction. The active fraction eluted from RESOURCE RPC was lyophilized, dissolved with DMSO, suspended in 50 mM MES pH 5.0/10% CH_3CN, and added to 1 ml of the cation exchange column RESOURCE S. Elution was carried out on a concentration gradient of 0 M-0.7 M NaCl. As a result, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was

detected in the 0.32 M-0.46 M NaCl fraction. The active eluate from RESOURCE S was lyophilized, dissolved with DMSO, suspended in 0.1% TFA/H_2O, and added to reversed-phase column C18 218TP5415 (Vydac), and elution was carried out on a concentration gradient of 20%-30% CH_3CN. As a result, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was detected in the three fractions 22.5%, 23%, and 23.5% CH_3CN (these active fractions are designated as P-1, P-2, and P-3) [Fig. 18]. Of the three active fractions, the 23.5% CH_3CN fraction (P-3) was lyophilized, dissolved with DMSO, suspended in 0.1% TFA/H_2O, and added to the reversed-phase column diphenyl 219TP5415 (Vydac), and elution was carried out on a gradient of 22%-25% CH_3CN. As a result, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was converged by recovered in one elution peak obtained with 23% CH_3CN [Fig. 19]. The peak activity fraction from the reverse-phased column diphenyl 219TP5415 was lyophilized, dissolved with DMSO, suspended in 0.1% TFA/H_2O, and added to the reversed-phase column μRPC C2/C18 SC 2.1/10 (Pharmacia), and elution was carried out on a gradient of 22%-23.5% CH_3CN. As a result, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was detected in the two peaks eluted with 23.0% and 23.2% CH_3CN [Fig. 20].

[Example 14]

Determination of the amino acid sequence of the peptide having the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells as purified from bovine hypothalamus

The amino acid sequence of the peptide (P-3)

having activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells as purified in Example 13 was determined. The fraction of peak activity from the reversed-phase μRPC C2/C18 SC 2.1/10 was lyophilized and dissolved in 20 μl of 70% CH_3CN and analyzed for amino acid sequence with the peptide sequencer (ABI.491). As a result, the sequence defined by SEQ ID NO:3 was obtained. However, the 7th and 19th amino acids were not determined by only the analysis of amino acid sequence.

[Example 15]

Preparation of the active substance (peptide) which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells as purified from bovine hypothalamus

Of the three active fractions obtained with Vydac C18 218TP5415 in Example 13, the active fraction (P-2) eluted with 23.0% CH_3CN was further purified. This active fraction was lyophilized, dissolved with DMSO, suspended in 0.1% TFA/dH_2O, and added to reversed-phase column diphenyl 219TP5415 (Vydac), and elution was carried out on a gradient of 21.0%-24.0% CH_3CN. As a result, activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was detected in a peak eluted with 21.9% CH_3CN. This fraction was lyophilized, dissolved with DMSO, suspended in 0.1% TFA/dH_2O, and added to reversed-phase μRPC C2/C18 SC 2.1/10 (Pharmacia), and elution was carried out on a CH_3CN gradient of 21.5%-23.0%. As a result, the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells converged in one peak eluted with 22.0% CH_3CN [Fig. 21].

[Example 16]

Determination of the amino acid sequence of the

peptide (P-2) purified from bovine hypothalamus which specifically promotes release of arachidonic acid metabolites from CHO-19P2 cells

The amino acid sequence of the peptide (P-2) having the activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells as purified in Example 15 was determined. The peak activity fraction from the reversed-phase column μRPC C2/C18 SC 2.1/10 was lyophilized, dissolved in 20 μl of 70% CH_3CN, and analyzed for amino acid sequence with the peptide sequencer (ABI, 492) (SEQ ID NO:4).

[Example 17]

Preparation of a poly(A)$^+$RNA fraction from bovine hypothalamus and synthesis of a cDNA

Using Isogen (Nippon Gene), total RNA was prepared from one animal equivalent of bovine hypothalamus. Then, using Fast Track (Invitrogen), a poly(A)$^+$RNA fraction was prepared. From 1 μg of this poly(A)$^+$RNA fraction, cDNA was synthesized using 3′ RACE system (GIBCO BRL) and Marathon cDNA amplification kit (Clontech) according to the manuals and dissolved in 20 and 10 μl, respectively.

[Example 18]

Acquisition of cDNA coding for the amino acid sequence established in Example 14

To obtain a cDNA coding for a polypeptide comprising the amino acid sequence established in Example 14, the acquisition of a base sequence coding for SEQ ID NO:1 was attempted in the first place. Thus, primers P5-1 (SEQ ID NO:35), P3-1 (SEQ ID NO:36), and P3-2 (SEQ ID NO:37) were synthesized. (In the Sequence Table, I represents inosine). Using 0.5 μl of the cDNA prepared by 3′ RACE in Example 17 as a template and EXTaq (Takara Shuzo Co., Japan) as DNA polymerase, 2.5 μl of accompanying buffer, 200 μM of accompanying dNTP, and primers P5-1 and P3-1 were added

each at a final concentration of 200 nM, with water added to make 25 µl, and after one minute at 94°C, the cycle of 98°C x 10 seconds, 50°C x 30 seconds, 68°C x 10 seconds was repeated 30 times. This reaction mixture was diluted 50-fold with tricine-EDTA buffer and using 2.5 µl of the dilution as a template and the primer combination of P5-1 and P3-2, the reaction was carried out in otherwise the same manner as described above. As the thermal cycler, Gene Amp 9600 (Perkin Elmer) was used. The amplification product was subjected to 4% agarose electrophoresis and ethidium bromide staining and a band of about 70 bp was cut out and subjected to thermal fusion, phenol extraction, and ethanol precipitation. The recovered DNA was subcloned into plasmid vector PCR™II according to the manual of TA Cloning kit (Invitrogen). The vector was then introduced into E. coli JM109 and the resultant transformant was cultured in ampicillin-containing LB medium. The plasmid obtained with an automatic plasmid extractor (Kurabo) was reacted according to the manual of Dye Terminator Cycle Sequencing Kit (ABI) and decoded with a fluorescent automatic DNA sequencer (ABI). As a result, the sequence shown in Fig. 22 was obtained and confirmed to be part of the base sequence coding for SEQ ID NO:1.

[Example 19]

Acquisition of a bioactive polypeptide cDNA by RACE using the sequence established in Example 18

First, for amplification (5′ RACE) of the sequence at 5′ end, the two primers PE (SEQ ID NO:38) and PDN (SEQ ID NO:39) were synthesized by utilizing the sequence shown in Fig. 22. The cDNA prepared using Marathon cDNA amplification kit in Example 17 was diluted 100-fold with tricine-EDTA buffer. Then, in the same manner as Example 2, a reaction mixture was prepared using 2.5 µl of the dilution and a combination

of the adapter primer AP1 accompanying the kit and the primer PE and after one minute at 94°C, the cycle of 98°C x 10 seconds and 68°C x 5 minutes was repeated 30 times. This reaction system was further diluted 50-fold with tricine-EDTA buffer and using 2.5 µl of the dilution as a template and the changed primer combination of AP1 and PDN, the reaction was conducted at 94°C for one minute, followed by 4 cycles of 94°C x 1 minute, 98°C x 10 seconds, 72°C x 5 minutes, 4 cycles of 98°C x 10 seconds, 70°C x 5 minutes, and 26 cycles of 98°C x 10 seconds, 68°C x 5 minutes. The amplification product was electrophoresed on 1.2% agarose gel and stained with ethidium bromide and a band of about 150 bp was cut out and centrifugally filtered through a centrifugal filter tube (Millipore), extracted with phenol, and precipitated from ethanol. The recovered DNA was subcloned into plasmid vector PCR™II according to the manual of TA Cloning Kit (Invitrogen). The vector was then introduced into E. coli JM109 and the resulting transformant was cultured and the sequence of the inserted cDNA fragment was analyzed as in Example 18. As a result, the sequence shown in Fig. 23 was obtained. Based on this sequence, primers FB (SEQ ID NO:40) and FG (SEQ ID NO:41) were synthesized and the 3′ sequence was cloned (3′ RACE). Using the same template as that for 5′ RACE in the same quantity and the combination of the accompanying adapter primer AP1 with the primer FC, PCR was carried out at 94°C for 1 minute, followed by 5 cycles of 98°C x 10 seconds, 72°C x 5 minutes, 5 cycles of 98°C x 10 seconds, 70°C x 5 minutes, and 25 cycles of 98°C x 10 seconds, 68°C x 5 minutes. Then, using 2.5 µl of a 50-fold dilution of this reaction mixture in tricine-EDTA buffer as the template and the combination of the accompanying primer AP2 with the primer FB, the reaction was further conducted at 94°C for one minute,

followed by 4 cycles of 98°C x 10 seconds, 72°C x 5 minutes, 4 cycles of 98°C x 10 seconds, 70°C x 5 minutes, and 27 cycles of 98°C x 10 seconds, 68°C x 5 minutes. The amplification product was electrophoresed on 1.2% agarose gel and stained with ethidium bromide and a band of about 400 bp was cut out and the DNA was recovered as in 5'-RACE. This DNA fragment was subcloned into plasmid vector pCR™II and introduced into E. coli JM109 and the sequence of the inserted cDNA fragment in the resulting transformant was analyzed. From the results of 5' RACE and 3' RACE, the DNA sequence [Fig. 24] coding for the complete coding region of the bioactive polypeptide defined by SEQ ID NO:1 was established. Thus, in Fig. 24 (a) and (b), the base^{134} is G, the base^{184} is T or C, and the base^{245} was T or C.

The cDNA shown in Fig. 24 was the cDNA encoding a polypeptide consisting of 98 amino acids. The fact that the amino acids in 1 - 22-positions comprise a cluster of hydrophobic amino acids taken together with the fact that the N-terminal region of the active peptide begins with Ser in 23-position as shown in Example 14 suggested that the amino acids 1-22 represent a secretion signal sequence. On the other hand, the Gly Arg Arg Arg sequence in 54-57 positions of the polypeptide was found to be a typical amino acid sequence motif which exists in the event of cleavage of a bioactive peptide. As it is the case with this cleavage motif, it is known that because of the presence of Gly, the C-terminus of the product peptide is frequently amidated.

The P-3 N-terminal sequence data of Example 14 and P-2 N-terminal sequence data in Example 16 coupled with this GlyArgArgArg sequence suggest that at least same of the bioactive peptides cut out from the polypeptide encoded by this cDNA are defined by SEQ ID NO:3, SEQ ID

NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10.

[Example 20]

Acquisition of a DNA fragment comprising the full coding region of bovine-derived bioactive polypeptide cDNA by PCR

Using the cDNA prepared with Marathon cDNA amplification kit in Example 17 as a template, a DNA fragment including the entire coding region of bioactive polypeptide cDNA was constructed. First, based on the sequence of cDNA elucidated in Example 19, two primers having base sequences defined by SEQ ID NO:42 and SEQ ID NO:43, respectively, were synthesized.

BOVF

5'-GTGTCGACGAATGAAGGCGGTGGGGGCCTGGC-3' (SEQ ID NO:42)

BOVR (24 mer)

5'-AGGCTCCCGCTGTTATTCCTGGAC-3' (SEQ ID NO:43)

BOVF contains the initiation codon of bioactive polypeptide cDNA and is a sense sequence corresponding to -2 - +22 (A of the initiation codon ATG being reckoned as +1) with restriction enzyme SalI site added. On the other hand, BOVR is an antisense sequence corresponding to +285 - +309 which includes the termination codon of bioactive polypeptide cDNA.

The PCR was conduced as follows. The cDNA prepared using Marathon cDNA amplification kit in Example 17 was diluted 100-fold in tricine-EDTA buffer and using 2.5 µl of the dilution, a reaction mixture was prepared as in Example 2 and subjected to 94°C x 1 minute, 3 cycles of 98°C x 10 seconds, 72°C x 5 minutes, 3 cycles of 98°C x 10 seconds, 70°C x 5 minutes, and 27 cycles of 98°C x 10 seconds, 68°C x 5 minutes. The amplification product was subjected to 2% agarose electrophoresis and ethidium bromide staining and a band of about 320 bp was cut out. The DNA was recovered and subcloned in plasmid vector pCR™II as in

Example 3. The vector was introduced into Escherichia coli JM109 to provide the transformant E. coli JM109/pBOV3. The sequence of the cDNA fragment inserted in the transformant was then analyzed. As a result, this DNA fragment was confirmed to be a fragment covering the entire coding region of the bioactive polypeptide cDNA.

[Example 21]

Synthesis of Ser-Arg-Ala-His-Gln-His-Ser-Met-Glu-Ile-Arg-Thr-Pro-Asp-Ile-Asn-Pro-Ala-Trp-Tyr-Ala-Gly-Arg-Gly-Ile-Arg-Pro-Val-Gly-Arg-Phe-NH2 (19P2-L31) SEQ ID NO: 97

1) Synthesis of Ser(Bzl)-Arg(Tos)-Ala-His(Bom)-Gln-His(Bom)-Ser(Bzl)-Met-Glu(OcHex)-Ile-Arg(Tos)-Thr(Bzl)-Pro-Asp(OcHex)-Ile-Asn-Pro-Ala-Trp(CHO)-Tyr(Br-Z)-Ala-Gly-Arg(Tos)-Gly-Ile-Arg(Tos)-Pro-Val-Gly-Arg(Tos)-Phe-pMBHA-resin

The reactor of a peptide synthesizer (Applied Biosystems 430A) was charged with 0.71 g (0.5 mmole) of commercial p-methyl-BHA resin (Applied Biosystems, currently Perkin Elmer). After wetting with DCM, the initial amino acid Boc-Phe was activated by the HOBt/DCC method and introduced into the p-methyl-BHA resin. The resin was treated with 50% TFA/DCM to remove Boc and make the amino group free and neutralized with DIEA. To this amino group was condensed the next amino acid Boc-Arg(Tos) by the HOBt/DCC method. After the absence of unreacted amino function was verified by ninhydrin test, a sequential condensation of Boc-Gly, Boc-Val, Boc-Pro, Boc-Arg(Tos), Boc-Ile, Boc-Gly, Boc-Arg(Tos), Boc-Gly, Boc-Ala, Boc-Tyr(Br-Z) was carried out. The Boc-Ala, Boc-Tyr (Br-Z), the condensation of which was found insufficient by ninhydrin test, was recondensed to complete the reaction. The resin was dried and a half of the resin was withdrawn. To the remainder, Boc-

Trp(CHO), Boc-Ala, Boc-Pro, Boc-Asn, Boc-Ile, Boc-Asp(OcHex), Boc-Pro, Boc-Thr(Bzl), Boc-Arg(Tos), Boc-Ile, Boc-Glu(OcHex), Boc-Met, Boc-Ser(Bzl), Boc-His(Bom), Boc-Gln, Boc-His(Bom), Boc-Ala, Boc-Arg(Tos), Boc-Ser(Bzl) were serially condensed and recondensed until sufficient condensation was confirmed by ninhydrin test. After introduction of the full sequence of amino acids of 19P2-L31, the resin was treated with 50% TFA/DCM to remove Boc groups on the resin and, then, dried to provide 1.28 g of the peptide resin.

2) Synthesis of Ser-Arg-Ala-His-Gln-His-Ser-Met-Glu-Ile-Arg-Thr-Pro-Asp-Ile-Asn-Pro-Ala-Trp-Tyr-Ala-Gly-Arg-Gly-Ile-Arg-Pro-Val-Gly-Arg-Phe-NH2(19P2-L31) Seq ID NO. 97

In a Teflon hydrogen fluoride reactor, the resin obtained in 1) was reacted with 3.8 g of p-cresol, 1 ml of 1,4-butanedithiol, and 10 ml of hydrogen fluoride at 0°C for 60 minutes. The hydrogen fluoride and 1,4-butanedithiol (1 ml) were distilled off under reduced pressure and the residue was diluted with 100 ml of diethyl ether, stirred, filtered through a glass filter, and the fraction on the filter was dried. This fraction was suspended in 50 ml of 50% acetic acid/H_2O and stirred to extract the peptide. After separation of the resin, the extract was concentrated under reduced pressure to about 5 ml and chromatographed on Sephadex G-25 (2 x 90 cm). Development was carried out with 50% acetic acid/H_2O and the 114 ml - 181 ml fraction was pooled and lyophilized to recover 290 mg of white powders containing 19P2-L31. The powders were applied to a reversed-phase column of LiChroprep RP-18 (Merck) and repeatedly purified by gradient elution using 0.1% TFA/H_2O and 0.1% TFA-containing 30% acetonitrile/H_2O. The fraction eluted at about 25% acetonitrile was pooled and lyophilized to provide 71 mg of white powders.

Mass spectrum $(M+H)^+$ 3574.645

HPLC elution time 18.2 min.

Column conditions

Column: Wakosil 5C18 (4.6 x 100 mm)

Eluent: A (0.1% TFA/H_2O)

B (0.1% TFA-containing 50 (% acetonitrile/H_2O)

Linear gradient elution from A to B (25 min.)

Flow rate: 1.0 ml/min.

[Example 22]

Synthesis of Ser-Arg-Ala-His-Gln-His-Ser-Met(O)-Glu-Ile-Arg-Thr-Pro-Asp-Ile-Asn-Pro-Ala-Trp-Tyr-Ala-Gly-Arg-Gly-Ile-Arg-Pro-Val-Gly-Arg-Phe-NH2(19P2-L31(O))

In 20 ml of 5% acetic acid/H_2O was dissolved 6 mg of synthetic 19P2-L31 and the Met only was selectively oxidized with 40 µl of 30% H_2O_2. After completion of the reaction, the reaction mixture was immediately applied to a reversed-phase column of LiChroprep RP-18 (Merck) for purification to provide 5.8 mg of the objective peptide.

Mass spectrum $(M+H)^+$ 3590.531

HPLC elution time 17.9 min.

Column conditions

Column: Wakosil 5C18 (4.6 x 100 mm)

Eluent: A (0.1% TFA/H_2O)

B (0.1% TFA-containing 50% aceto nitrile/H_2O)

Linear gradient elution from A to B (25 min.)

Flow rate: 1.0 ml/min.

[Example 23]

Synthesis of Thr-Pro-Asp-Ile-Asn-Pro-Ala-Trp-Tyr-Ala-Gly-Arg-Gly-Ile-Arg-Pro-Val-Gly-Arg-Phe-NH2(19P2-L20)

To the resin subjected to condensations up to Boc-Tyr(Br-Z) in Example 21-1) was further condensed Boc-Trp(CHO), Boc-Ala, Boc-Pro, Boc-Asn, Boc-Ile, Boc-

Asp(OcHex), Boc-Pro, Boc-Thr(Bzl) serially in the same manner to provide 1.14 g of Boc-Thr(Bzl)-Pro-Asp(OcHex)-Ile-Asn-Pro-Ala-Trp(CHO)-Tyr(Br-Z)-Ala-Gly-Arg(Tos)-Gly-Ile-Arg(Tos)-Pro-Val-Gly-Arg(Tos)-Phe-pMBHA-resin. This resin was treated with hydrogen fluoride and columnwise purified in the same manner as Example 21-2) to provide 60 mg of white powders.

Mass spectrum $(M+H)^+$ 2242.149

HPLC elution time 10.4 min.

Column conditions

Column: Wakosil 5C18 (4.6 x 100 mm)

Eluent: A (0.1% TFA-containing 15% aceto nitrile/H_2O)

B (0.1% TFA-containing 45% aceto nitrile/H_2O)

Linear gradient elution from A to B (15/min.)

Flow rate: 1.0 ml/min.

[Example 24]

Determination of arachidonic acid metabolites-releasing activity of synthetic peptide (19P2-L31)

The activity of the peptide (19P2-L31) synthesized in Example 21 to specifically release arachidonic acid metabolites from CHO-19P2 cells was assayed in the same manner as Example 11. The synthetic peptide was dissolved in degassed dH_2O at a concentration of 10^{-3}M and diluted with 0.05% BSA-HBSS and the activity to promote release of arachidonic acid metabolites from CHO-19P2 cells at each concentration was assayed using the amount of [^{3}H]arachidonic acid metabolites as the indicator. As a result, concentration-dependent arachidonic acid metabolite-releasing activity was detected over the range of 10^{-12}M - 10^{-6}M [Fig. 25]. When the arachidonic acid metabolite-releasing activity of peptide 19P2-L31(O), i.e. the methionine-oxidation product of 19P2-L31 synthesized in Example 22, was compared with that of 19P2-L31, it was found that the

activity of 19P2-L31(O) was equivalent to the activity of 19P2-L31 as can be seen from Fig. 26.

[Example 25]

Determination of arachidonic acid metabolites-releasing activity of synthetic peptide (19P2-L20)

The activity of the synthetic equivalent (19P2-L20) of natural peptide P-2 as synthesized in Example 23 to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells was determined as in Example 11. Thus, the synthetic peptide was dissolved in degassed dH_2O at a final concentration of 10^{-3}M and this solution was serially diluted with 0.05% BAS-HBSS. The activity to specifically promote release of arachidonic acid metabolites from CHO-19P2 cells at each concentration was assayed using the amount of [^{3}H]arachidonic acid metabolites as the indicator.

As a result, concentration-dependent arachidonic acid metabolite-releasing activity was detected over the range of 10^{-12} - 10^{-6}M in nearly the same degree as 19P2-L31 [Fig. 27].

[Example 26]

Analysis of the coding region base sequence of bovine genomic DNA

pBOV3 was digested with restirction enzyme EcoRI and after fractionation by agarose gel electrophoresis, the DNA corressponding to the cDNA fragment was recovered to prepare a probe. This DNA was labeled with ^{32}P using a multiprime DNA labeling kit (Amersham). About 2.0×10^6 phages of Bovine Genomic Library (Clontech BL1015j) constructed using cloning vector EMBL3 SP6/T7 and Escherichia coli K802 as the host were seeded in an LB agar plate and cultured overnight for plaque formation. The plaques were transferred to a nitrocellulose filter and after alkaline modification and neutralization, heat-treated (80°C, 2 hours) to inactivate the DNA. This filter was

incubated with the labeled probe in 50% formamide-Hybri buffer (50% formamide, 5 x Denhardt solution, 4 x SSPE, 0.1 mg/ml heat-denatured salmon sperm DNA, 0.1% SDS) at 42°C overnight for hybridization. After this hybridization, the filter was washed with 2 x SSC, 0.1% SDS at room temperature for 1.5 hours, and further washed in the same buffer at 55°C for 30 minutes. Detection of the clone hybridizing with the probe was carried out on Kodak X-ray film (X-OMAT™AR) after 4 days of exposure using a sensitization screen at -80°C. After development of the film, the film was collated with plate positions and the phages which had hybridized were recovered. Then, plating and hybridization were repeated in the same manner for cloning of the pharges.

The cloned phages were prepared on a large scale by the plate lysate method and the phage DNA was extracted. Then, cleavage at the restriction enzyme SalI and BamHI cleavage sites at both ends of the cloning site of the vector and detection of the inserted fragment derived from bovine genomic DNA was carried out by 1.2% agarose gel electrophoresis [Fig. 28]. As a result, in the case of BamHI digestion, 3 fragments were detected in addition to the bands derived from the phages. In the case of SalI digestion, one band overlapping the phage band was detected. The SalI-digested fragment being considered to harbor the full length and in order to subclone this fragment into a plasmid vector, it was ligated to BAP (*E. coli*-derived alkaline phosphatase)-treated plasmid vector pUC18 (Pharmacia) and introduced into *E. coli* JM109. From this microorganism, a genome-derived SalI fragment-inserted plasmid DNA was prepared on a production scale and the base sequence in the neighborhood of its coding region was analyzed using Perkin Elmer Applied Biosystems 370A fluorecent

sequencer and the same manufacturer's kit. As a result, the sequence shown in Fig. 29 was obtained. Comparison with the coding region of cDNA reveals that because of its being derived from genomic DNA, the coding region is divided in two by a 472 bp intron [Fig. 30]. Fig. 31 and SEQ ID NO:44 present the amino acid sequence predicted from this bovine genome coding region (excluding the intron region).

[Example 27]

Preparation of rat medulla oblongata poly(A^+)RNA fraction and synthesis of cDNA

Using Isogen (Nippon Gene), total RNA was prepared from the dorsal region of rat medulla oblongata and using FastTrack (Invitrogen), poly$(A)^+$RNA fraction was prepared. To 5 µg of this poly$(A)^+$RNA was added the primer random DNA hexamer (BRL) and using Moloney mouse leukemia reverse transcriptase (BRL) and the accompanying buffer, complementary DNA was synthesized. The reaction product was precipitated from ethanol and dissolved in 12 µl of DW. In addition, from 1 µg of this poly$(A)^+$RNA, a cDNA was synthesized using Marathon cDNA amplification kit (Clontech) according to the manual and dissolved in 10 µl of DW.

[Example 28]

Acquisition of rat bioactive polypeptide cDNA by RACE

To obtain the full coding region of rat bioactive polypeptide cDNA, an experiment was performed in the same manner as the acquisition of bovine cDNA. First, PCR was carried out using the same primers P5-1 (SEQ ID NO:35) and P3-1 (SEQ ID NO:36) as used in Example 18 as primers and the complementary DNA synthesized in Example 27 using the primer random DNA hexamer (BRL) and Moloney mouse leukemia reverse transcriptase (BRL) as a template. The reaction system was composed of 1.25 µl of the template cDNA, 200 µM of dNTP, 1 µM each

of the primers, ExTaq (Takara Shuzo Co., Japan) as DNA polymerase, and 2.5 µl of the accompanying buffer, with a sufficient amount of water to make a total of 25 µl. The reaction was carried out at 94°C for 1 minute, followed by 40 cycles of 98°C x 10 seconds, 50°C x 30 seconds, and 72°C x 5 seconds, and the reaction mixture was then allowed to stand at 72°C for 20 seconds. The thermal cycler used was GeneAmp2400 (Perkin Elmer). The amplification product was subjected to 4% agarose electrophoresis and ethidium bromide staining and the band of about 80 bp was cut out. Then, in the manner described in Example 19, the DNA was recovered, subcloned into plasmid vector pCR™II, and introduced into E. coli JM109, and the inserted cDNA fragment was sequenced. As a result, a partial sequence of rat bioactive polypeptide could be obtained. Based on this sequence, two primers, namely RA (SEQ ID NO:75) for 3' RACE and RC (SEQ ID NO:76) for 5' RACE were synthesized and 5' and 3' RACEs were carried out.

RA:5'-CARCAYTCCATGGAGACAAGAACCCC-3'
(where R means A or G; Y means T or G) (SEQ ID NO:75)
RC:5'-TACCAGGCAGGATTGATACAGGGG-3'
(SEQ ID NO:76)

As a template, the template synthesized using Marathon cDNA amplification kit (Clontech) in Example 27 was diluted 40-fold with the accompanying tricine-EDTA buffer and 2.5 µl of the dilution was used. As primers, RA and the adapter primer AP1 accompanying the kit were used for 3' RACE, and RC and AP1 for 5' RACE. The reaction mixture was prepared in otherwise the same manner as above. The reaction conditions were 94°C x 1 minute, 5 cycles of 98°C x 10 seconds, 72°C x 45 seconds, 3 cycles of 98°C x 10 seconds, 70°C x 45 seconds, and 40 cycles of 98°C x 10 seconds, 68°C x 45 seconds. As a result, a band of about 400 bp was obtained from 3' RACE and bands of about 400 bp and 250

bp from 5′ RACE. These bands were recovered in the same manner as above and using them as templates and the primers used in the reaction, sequencing was carried out with Dye Terminator Cycle Sequencing Kit (ABI). As a result, the sequence up to poly A could be obtained from the region considered to be the 5′ noncoding region.

[Example 29]

Acquisition of the full-length cDNA of rat bioactive polypeptide by PCR

Based on the sequence obtained in Example 28, two primers, viz. rF for the region including the initiation codon (SEQ ID NO:77) and rR for the 3′ side from the termination codon (SEQ ID NO:78), were synthesized to amplify the fragment including the full-length cDNA.

rF:5′-GGCATCATCCAGGAAGACGGAGCAT-3′ (SEQ ID NO:77)

rR:5′-AGCAGAGGAGAGGGAGGGTAGAGGA-3′ (SEQ ID NO:78)

Using the cDNA prepared using Moloney mouse leukemia reverse transcriptase in Example 27 as a template and ExTaq (Takara Shuzo Co., Japan), PCR was carried out by repeating 40 cycles of 95°C x 30 seconds, 68°C x 60 seconds. The amplification product was subjected to agarose electrophoresis and ethidium bromide staining and a band of about 350 bp was cut out. The DNA was recovered, subcloned into plasmid vector pCR™II, and introduced into E. coli JM109 as in Example 19. The plasmid was extracted from the transformant and the base sequence was determined. As a result, E. coli JM 109/pRAV3 having the full-length cDNA of rat bioactive polypeptide was obtained [Fig. 32].

[Example 30]

Synthesis of cDNA from the human total brain ply(A)$^+$RNA fraction

From 1 μg of human total brain poly(A)$^+$RNA

fraction (Clontech), cDNA was synthesized with Marathon cDNA amplification kit (Clontech) according to the manual and dissolved in 10 µl. In addition, the random DNA hexamer (BRL) was added as primer to 5 µg of the same poly(A)$^+$RNA fraction and using Moloney mouse leukemia reverse transcriptase (BRL) and the accompanying buffer, complementary DNA was synthesized. The reaction product was precipitated from ethanol and dissolved in 30 µl of TE.

[Example 31]

Acquisition of human bioactive polypeptide cDNA by RACE

From the amino acid sequence of rat bioactive polypeptide established in Example 28 [Fig. 33], the well-preserved regions of rat and bovine polypeptides were selected and the following 3 primers R1, (SEQ ID NO:79), R3 (SEQ ID NO:80), and R4 (SEQ ID NO:81) were synthesized. Then, amplification of the region flanked by them was attempted by PCR using human cDNA as a template. Referring to Fig. 33, bovine. aa represents the amino acid sequence of bovine polypeptide, bovine. seq represents the base sequence of the DNA coding for bovine polypeptide, and rat. seq represents the base sequence of the DNA coding for rat polypeptide.

R1:5'-ACGTGGCTTCTGTGCTTGCTGC-3' (SEQ ID NO:79)
R3:5'-GCCTGATCCCGCGGCCCGTGTACCA-3' (SEQ ID NO:80)
R4:5'-TTGCCCTTCTCCTGCCGAAGCGGCCC-3' (SEQ ID NO:81)

The cDNA prepared using Marathon cDNA amplification kit (Clontech) in Example 30 was diluted 30-fold with tricine-EDTA buffer and 0.25 µl of the dilution was used as a template. The reaction mixture was composed of 200 µM of dNTP, 0.2 µM each of the primers R1 and R4, a 50:50 mixture of Taq Start Antibody (Clontech) and DNA polymerase ExTaq (Takara Shuzo Co., Japan), 2.5 µl of the accompanying buffer, and a sufficient amount of water to make a total of 25

µl. The reaction conditions were 94°C x 1 minute, followed by 42 cycles of 98°C x 10 seconds, 68°C x 40 seconds, and 1 minute of standing at 72°C. Then, using 1 µl of a 100-fold dilution of the above reaction mixture in tricine-EDTA buffer as a template, the same reaction mixture as above except that the primer combination was changed to R1 and R3 was prepared and PCR was carried out in the sequence of 94°C x 1 minute and 25 cycles of 98°C x 10 seconds, 68°C x 40 seconds. The amplification product was subjected to 4% agarose electrophoresis and ethidium bromide staining. As a result, a band of about 130 bp was obtained as expected. This band was recovered in the same manner as in Example 28 and using the recovered fragment as a template, sequencing was carried out with Dye Terminator Cycle Sequencing Kit (ABI). As a result, a partial sequence of human bioactive polypeptide could be obtained. Therefore, based on this sequence, primers HA (SEQ ID NO:82) and HB (SEQ ID NO:83) were synthesized for 3' RACE and primers HE (SEQ ID NO:84) and HF (SEQ ID NO:85) for 5' RACE and 5' and 3' RACEs were carried out.

HA:5'-GGCGGGGGCTGCAAGTCGTACCCATCG-3' (SEQ ID NO:82)
HB:5'-CGGCACTCCATGGAGATCCGCACCCCT-3' (SEQ ID NO:83)
HE:5'-CAGGCAGGATTGATGTCAGGGGTGCGG-3' (SEQ ID NO:84)
HF:5'-CATGGAGTGCCGATGGGTACGACTTGC-3' (SEQ ID NO:85)

As the template, 2.5 µl of a 20-fold dilution of the cDNA prepared in Example 30 in tricine-EDTA buffer was used. For the initial PCR, reaction mixtures were prepared in the same manner as above except that HA and adapter primer AP1 were used for 3' RACE and HE and AP1 for 5' RACE. The reaction sequence was 94°C x 1 minute, 5 cycles of 98°C x 10 seconds, 72°C for 35 seconds, 5 cycles of 98°C x 10 seconds, 70°C x 35 seconds, and 40 cycles of 98°C x 10 seconds, 68°C x 35 seconds. Then, using 1 µl of a 100-fold dilution of

this reaction mixture in tricine-EDTA buffer as a template, a second PCR was carried out in the same cycles as the first PCR. However, the reaction mixture was prepared using primers HB and AP1 for 3' RACE or HF and AP2 for 5' RACE and Klen Taq (Clontech) as DNA polymerase and the accompanying buffer. As a result, a band of about 250 bp was obtained from 3' RACE and a band of about 150 bp from 5'-RACE. These bands were sequenced by the same procedure as above and using them in combination with the partial sequence obtained previously, the sequence from the region presumed to be 5'-noncoding region to polyA of human bioactive polypeptide was obtained.

[Example 32]

Acquisition of human bioactive polypeptide full-length cDNA by PCR

Based on the sequence obtained in Example 31, two primers 5H (SEQ ID NO:86) and 3HN (SEQ ID NO:87) were synthesized for amplification of a fragment including full-length cDNA.

5H:5'-GGCCTCCTCGGAGGAGCCAAGGGATGA-3' (SEQ ID NO:86)

3HN:5'-GGGAAAGGAGCCCGAAGGAGAGGAGAG-3' (SEQ ID NO:87)

Using 2.5 µl of the cDNA prepared using Moloney mouse leukemia reverse transcriptase (BRL) in Example 30 as a template and the reaction mixture prepared using Klen Taq DNA polymerase (Clontech), the PCR reaction was conducted in the sequence of 94°C x 1 minute and 40 cycles of 98°C x 10 seconds, 68°C x 30 seconds. The fragment of about 360 bp obtained was recovered and subcloned (pCR™ 2.1 was used as the vector) in otherwise the same manner as Example 29. The plasmid was recovered and its base sequence was determined. As a result, E. coli JM109/pHOV7 harboring the human bioactive polypeptide full-length cDNA was obtained [Fig. 34]. In regard to the amino acid sequence of the translation region, a comparison was

made between this human bioactive polypeptide and the bovine polypeptide shown in Example 20 or the rat polypeptide in Example 29 [Fig. 35].

[Example 33]

An orphan G-protein coupled receptor, UHR-1, has been cloned from rat hypothalamic suprachiasmic nuclei, and its nucleotide sequences have been reported (Biochemical and Biophysical Research Communications, vol. 209, No.2, pp606-613, 1995., Genbank Accession Number: S77867). A protein coded by UHR-1 showed 91.6% identity over 359 amino acids with that of phGR3, suggesting UHR-1 is a counterpart of hGR3. To confirm this we cloned a cDNA for UHR-1 coding regions and established a CHO cells stably expressing UHR-1 as described below. Poly(A)$^+$ RNA was prepared from rat anterior pituitary using a FastTrack™ Kit (Invitrogen Co.), and cDNA was synthesized from 0.2 μg of this with Takara RNA PCR Kit (Takara). The cDNA was dissolved in 10 μl of distilled water, and used as a template for the following PCR. To isolate UHR-1 cDNA, two primers, namely 5'-GTTCACAG(GTCGAC)ATGACCTCAC-3' [SEQ ID NO:95] (UHF), and 5'-CTCAGA(GCTAGC)AGAGTGTCATCAG-3' [SEQ ID NO:96] (UHR), were synthesized on the basis of the sequence of UHR-1 submitted to Genbank (Accesion Number: S77867). In these primers, GTCGAC and GCTAGC indicate the SalI and NheI site respectively. Ex Taq (Takara) was admixed with an equal amount of Taq Start Antibody (Clontech Laboratories, Inc.) to prevent amplification of nonspecific products and primer dimers. Reaction mixture was prepared by adding 5 μl of the buffer attached to Ex Taq, 4 μl of dNTPs, 1 μl of the mixed solution of Ex Taq and Taq Start Antibody, and 1 μl of 50 μM each primers. The cDNA was diluted to one fifth with distilled water, and an aliguot (5 μl) was added to the reaction mixture. PCR conditions were as follows: denatured at 95°C for 2 minutes,

followed by 27 cycles at 95°C for 30 seconds, 65°C for 30 seconds and 72°C for 1 minutes, and after these cycles at 72°C for 7 minutes.

The PCR products were separated with 1.2% agarose gel and stained with ethidium bromide. Slices of agarose gel containing the band about 1.1 kbp were cut out with razor blade, and then filtered using an Ultra Free filter unit (Millipore). The eluent was extracted with phenol: chloroform and precipitated in ethanol. The amplified DNA was subcloned into pCR™II with a TA cloning Kit (Invitrogen Co.), and then introduced into E. coli JM109 competent cells. Transformants were selected in LB (Luria-Bertani) agar culture medium containing ampicillin, IPTG (isopropylthio-beta-D-galactoside), and X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside). The individual clones were cultured in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo) to prepare plasmid DNAs respectively. Sequencing was carried out with a ABI PRISM Dye Terminator Cycle Sequencing Kit FS (Perkin-Elmer), and an ABI automatic sequencer. In the Fig. 52, underlines indicate the sequences corresponding to the parts of primer sequences. Double-lined bases indicate the base substitution compared with the sequence data reported, and one of these substitutions was accompanied by an amino acid substitution from ^{289}Leu(CTC) to ^{289}Val(GTC). A plasmid, pCRII-UHR-1, containing the UHR-1 cDNA fragment was thus constructed.

UHR-1 cDNA expression plasmid was prepared as follows. First, pCRII-UHR-1 was digested with NheI and SalI. The resultant fragment of about 1.1 kbp was separated through electrophoresis using a 1.2% agarose gel and precipitated as above. The DNA fragment was then ligated into the NheI-SalI site of pAKKO-111H, with a Ligation System (Takara). A resultant

expression plasmid, pAKKO-UHR-1 was introduced into E. coli JM109.

CHO dhfr$^-$ cells were grown in 10 cm diameter Petri dishes at the cell number of $1x10^6$, and cultured at 37°C for 24 hours in α-MEM containing 10% of fetal bovine serum. The expression plasmid (20 μg) was introduced into the cells by a liposome method using a Gene Transfer (Nippon Gene). After 24 hours from the introduction, the medium was substituted with fresh one. After additional 24 hour incubation, the culture medium was changed to a Selection medium, α-MEM without nucleosides containing 10% of dialyzed fetal bovine serum. Culture was carried out until cells growing in the Selection medium were obtained. CHO-UHR-1 which highly expressed UHR-1 was thus established.

[Example 34]

Radioiodination of 19P2-L31 and receptor binding experiments

19P2-L31 was radioiodinated with [^{125}I]-Bolton-Hunter Reagent (NEN.Dupont; NEX-120) as follows. Two hundred microliter of [^{125}I]-Bolton-Hunter Reagent was dried in a 500 μl Eppendorf tube with N_2 gas. The dried reagent was dissolved in 2 μl of acetonitrile, and then mixed with 4 ml of 50 mM phosphate buffer (pH 8.0) and 4 μl of 19P2-L31 $3x10^{-4}$M. The mixture was incubated at room temperature for 40 min and the reaction was stopped by adding 5 μl of 1.0 M glycine. The all reaction mixture was diluted with 300 μl of 18% acetonitrile and injected onto reverse-phase HPLC column TSK gel ODS-80TM (4.6x100mm; TOSO). The radioiodinated 19P2-L31 was eluted with a linear gradient of acetonitrile concentration from 18 to 32.4% in 0.1% teifluoroacetic acid for 24 min at a flow rate of 1 ml/min. The peak fraction of radioiodinated 19P2-L31 was collected and diluted with twice volume of 50 mM Tris-HCl(pH7.5) containing 0.1% BSA and 0.05% CHAPS,

and then stored at -20°C.

Receptor binding experiments were performed with [^{125}I]-19P2-L31 as follows. As receptor-expressing CHO cells, CHO-19P2-9; mono-clone of CHO-19P2, CHO-UHR-1, and mock CHO were used in this experiment. CHO-19P2-9 cells are ones selected from CHO-19P2 cells by ultradilution technique using 96-well microplate as clone which indicated stronger arachidonic acid metabolic-release promoting reaction by 19P2-L31. The mock CHO cells are ones for control which were transformed with expression vector pAKKO alone. These cells cultured in flasks for culturing tissues were harvested with 5 mM EDTA/PBS, and then resuspended in HBSS containing 0.05% BSA and 0.05% CHAPS at $0.5x10^7$ cells/ml. The cell suspensions were incubated with 200 pM [^{125}I]-19P2-L31 for 2.5 hr at room temperature in a 100 µl total volume. The reaction mixture were diluted with 2 ml of an ice-cold beffer (50 mM Tris-HCl pH7.5 containing 5 mM EDTA, 0.05% BSA, and 0.05%CHAPS) and immediately filtered though glass filters GF/F (Whattman) which were pre-wetted with the buffer containing 0.3% polyethylenimine. The glass filters were subjected to γ-counting. Non-specific binding was determined in the presence of 200 nM unlabeled 19P2-L31.

[Fig. 36] shows receptor binding experiments with [^{125}I]-19P2-L31 on live cells.

Specific binding of [^{125}I]-19P2-L31 was detected on CHO cells which were expressed with hGR3 and rat homolog UHR-1 respectively. The experiments were performed in triplicate. These results show that the proteins encoded by hGR3 and UHR-1 is functioning as the specific receptor of 19P2-L31.

[Example 35]

Release of arachidonic acid metabolites from CHO-19P2-9 and CHO-UHR1 by 19P2-L31

Same as described in Example 11, the release activity of arachidonic acid metabolite was measured on CHO-19P2-9 and CHO-UHR1 and mock CHO.

[Fig. 37] shows the release activity of arachidonic acid metabolite on CHO-19P2-9 and CHO-UHR1 by 19P2-L31.

On CHO cells which were expressed with rat homolog UHR1, the release activity of arachidonic acid metabolite was detected same as CHO-19P2-9. The experiments were performed in duplicate. These results show that the protein encoded by UHR-1 is functioning as the specific receptor as well as hGR3.

[Example 36]

Quantification of rat 19P2 ligand and rat UHR-1 mRNA, BBRC, 209,606-613, 1995) by RT-PCR

(1) Preparation of poly(A)+RNA and cDNA synthesis from rat tissues.

Poly(A)+RNA was isolated from a variety of tissues in rats (Wister strain, male, 8 weeks old) by homogenization with Isogen (Nippon Gene) followed by an oligo (dT)-cellulose chromatography (Pharmacia). One μg of poly(A)+RNA was treated with DNase I (Amplification grade, GibcoBRL) to eliminate the contamination of genomic DNA. DNase I was inactivated by the addition of 25 mM EDTA solution at 65°C. Then RNA (160 ng) was reverse-transcribed in 40μl of a reaction miexture containing 10 mM of Tris-HCl (pH 8.3), 2.5 μM of random hexamers (Takara), 0.4 mM of each dNTP, and 10 U of AMV reverse transcriptase XL (Takara). The samples were incubated at 30°C for 10 min followed by 42°C for 1h, then 99°C for 5 min to stop the reaction. The reaction mixture was purified by ethanol precipitation, and then the cDNA was diluted to 40μl with tricine-EDTA buffer (correspond to 4 ng poly(A)+RNA/μl).

(2) Construction of positive control plasmid vectors

Rat glycerolardehyde-3-phasphate-dehydrogenase (G3PDH) and rat UHR-1 cDNAs were isolated from rat pituitary tumor cell line GH_3 by means of RT-PCR Poly(A)+RNA of GH_3 was prepared by FastTrack (Invitrogen), and cDNA was synthesized as Example 36(1). Oligonucleotide primers used for the amplification are as follows: rat G3PDH amplification primer set (Clontech), rRECF(5'-CCTGCTGGCCATTCTCCTGTCTTAC-3') (SEQ ID NO:88) and rRECR(5'-GGGTCCAGGTCCCGCAGAAGGTTGA-3') (SEQ ID NO:89) for UHR-1. The fragments amplified from GH_3 cDNA were subcloned with a TA cloning Kit (Invitrogen). The recombinant vectors were introduced into E. coli JM109. The transformant clones were cultured in a LB culture medium containing ampicillin, and the plasmid DNAs were prepared with a Quiagen Plasmid Midi Kit (Quiagen). The plasmid of rat ligand polypeptide was prepared from E. coli JM109/pRAV3 which was deposited.

(3) Quantification RT-PCR

cDNA and plasmid DNA prepared in (1) and (2) above were diluted with distilled water to adequate concentrations and used as templates of quantitative RT-PCR. G3PDH, UHR-1, and ligand polypeptide cDNA fragments were amplified using human G3PDH amplimer (Clontech), rRECF and rRECR, and r19F(5'-GAAGACGGAGCATGGCCCTGAAGAC-3') (SEQ ID NO:91) and r19R(5'-GGCAGCTGAGTTGGCCAAGTCCAGT-3') (SEQ ID NO:91), respectively. Each reaction sample contained 100 μM of dNTP mixture, 200 nM of each primer, 4 μl of template DNA, 0.25 μl of 50x KlenTaq DNA polymerase mix (Clontech), and 2.5 μl of the buffer attached to KenTaq DNA polymerase mix in a final volume of 25 μl. PCR conditions for G3PDH were as follows: denatured at 94°C for 1 min, followed by 26 cycles at 98°C for 10 sec, at 65°C for 20 sec, and at 72°C for 40 sec. PCR conditions for UHR-1 and ligand polypeptide were as

follows: denatured at 94°C for 1 min, followed by 34 cycles at 98°C for 10 sec, and at 68°C for 25 sec. An aliquot 5 µl of each RT-PCR product was separated with 4% Nusieve 3:1 agarose gel (F.M.C.) electrophoresis and stained with ethidium bromide. The bands were quantified using a densitometry program (Advanced American Biotechnology).

The results measured the expression levels of UHR-1 and ligand polypeptide mRNA in the tissues were shown in Fig. 38 and 39 respectively. UHR-1 and ligand polypeptide mRNA were detected in all the tissues tested. The highest level of UHR-1 mRNA expression was detected in the pituitary, and moderate expression levels in the brain, whereas poorly expressed in peripheral tissues except for the adrenal glands. Ligand polypeptide mRNA expressed mainly in the hypothalamus and dosal medulla among brain regions, and expressed comparatively high levels in the lung, thymus, pancreas, kidney, adrenal glands, and testis. These results show that the UHR-1 and ligand polypeptide play a significant role for the regulation of function in various tissues.

[Example 37]

Effect of 19P2-L31 on glucose-induced increase in plasma insulin concentration

Male Wistar rats (8-10w) were anesthetized by i.p. injection of pentobarbital (65 mg/kg). Glucose alone (86 mg/rat) or glucose and 19P2-L31 (675 pmol, 2.25 nmol, 6.75 nmol and 67.5 nmol/rat) were administered by bolus injection in the jugular vein. Blood samples were withdrawn from the contralateral vein. Plasma insulin concentration was determined with a radioimnunoassay kit (Amersham).

Administration of 19P2-L31 at the doses of 675 pmol, 2.25 nmol, and 6.75 nmol partially inhibited glucose-induced sharp increase (the first phase) in

plasma insulin concentration at 2 min postinjection and the blunt increase (the second phase) after 6 min postinjection. It completely inhibited the first and second phase of increase in insulin concentration at the dose of 67.5 nmol [Fig. 40].

[Example 38]

Effects of ligand polypeptide on motor activity of mouse

The effects of administration of 19P1-L31 to mouse lateral ventricle on motor activity were studied. The mature ICR male mice (weight at operation: about 35 g) were anesthetized by intraperitoneal administration of 50 mg/kg of pentobarbital, and then fixed on a stereotaxic apparatus. The skull of a said mouse was exposed, then a hole was made by dental drill for guide-cannulization into the left lateral ventricle. The tip of a stainless-steel guide-cannula (24G, length: 5 mm) for drug injection to lateral ventricle, was inserted to the position of AP: +0.6 mm (from bregma), L: left 1 mm and H: -1 mm (from dura matter). The guide-cannula was fixed onto the skull with adhesive. The cannula-implanted mice were housed as described above and were used for behavioral analysis at least 3 days after the operation.

Motor activity such as spontaneous motor activity and rearing was measured while each mouse was in a transparent acrylic cage (24 x 37 x 30 cm) within a soundproofed, illuminated (light up: at 6-18 o'clock) box. Tap water and laboratory chow were available ad libitum. Motor activity was measured by means of a Supermex (Muromachi Kikai). Drugs and PBS were administered at 2:30±30 p.m. At the administration, a stainless-steel micro-injection cannula (30G, length: 6 mm) was inserted into the guide-cannula. The micro-injection cannula was connected to a microsyringe pump with Teflon tube, and injection of PBS or a peptide

dissolved in PBS lasted for 2 minutes at a speed of 2 μl/min. The micro-injection cannula was withdrawn after over a period of 2 minutes from end of injection, then motor activity was meausred.

The results are expressed as a mean ± S.E.M. Student's t test was used to determine the significance of differences between values from the mice treated with a peptide and the PBS-injected controls. For the purpose of this analysis, $p<0.05$ was assumed to be the minimal level of significance.

As shown in [Fig. 41], administration of 10 nmol of 19P2-L31 caused a significant increase in spontaneous motor activity at 70-105 min after injection. Rearing behavior also showed significant variation. While the administration of 1 nmol of 19P2-L31 did not cause statistically significant change of spontaneous motor activity, rearing behavior showed a significant decrease at only 105 min after injection [Fig. 42]. The administration of 0.1 nmol of 19P2-L31 caused a significant increase at 25 min, 40 min and 70 min after injection. In that case, rearing behavior showed an increasing tendency similarly to spontaneous motor activity, however that was not statistically significant [Fig. 43]. The administration of 0.01 nmol of 19P2-L31 caused a significant increase at 20 min and 40 min after injection. In that case, rearing behavior showed an increasing tendency similarly to spontaneous motor activity, however that was not statistically significant [Fig. 44].

[Example 39]

Effects of ligand polypeptide on reserpine-induced hypothermia in mice

The mature ICR male mice (weight at operation: about 35 g) were anesthetized by administration of pentobarbital (50 mg/kg, i.p.), and then fixed on stereotaxic apparatus. The skull of a said mouse was

exposed, then a hole was made by dental drill for guide-cannulization into the left lateral ventricle. The tip of a stainless-steel guide-cannula (24G, length: 5 mm) for drug injection to lateral ventricle, was inserted to the position of AP: +0.6 mm (from bregma), L: left 1 mm and H: -1 mm (from dura matter). The guide-cannula was fixed onto the skull with adhesive. The cannula-implanted mice were housed as described above and were used for measurements of body temperature at least 3 days after the operation. Reserpine (Apoplon; Daiichi Pharmaceutical) was administered to mice at a dose of 3 mg/kg, s.c., and after 15 hours, each mouse was placed in a cage for the measurement. Then a stainless-steel micro-injection cannula (30G, length: 6 mm) was inserted into the guide-cannula. The micro-injection cannula was connected to a microsyringe pump with Teflon tube, and injection of PBS or a peptide dissolved in PBS lasted for 2 minutes at a speed of 1 µl/min. The micro-injection cannula was withdrawn after over a period of 2 minutes from end of injection, then the temperature in rectum was measured.

The results are expressed as a mean ± S.E.M. Student's t test was used to determine the significance of differences between values from the mice treated with a peptide and the PBS-injected controls. For the purpose of this analysis, $p<0.05$ was assumed to be the minimal level of significance.

As shown in [Fig. 45], body temperature which was lowered by reserpine increased significantly after a 10 nmol injection of 19P2-L31 in contrast to the control which PBS were administered. This increase of body temperature reached a maximum level at 45 min after administration of the peptide. On the other hand, there was no statistically significant difference in temperature variation between 1 nmol of 19P2-L31 and

the PBS-injected control throughout the experimental period.

[Example 40]

Effects of ligand polypeptide on blood pressure in rats

The inventors explored the influence of injection of 19P2-L31 into the area postrema of medula oblongata on blood pressure. Mature male Wistar rats (body weights at operation: ca 300 g) were anesthetized with pentobarbital 50 mg/kg i.p. and each animal was immobilized in a rat brain stereotaxic apparatus. The incisor bar was lowered by 3.3 mm from the interaural line. The skull was exposed, and using a dental drill a hole was made on the skull for implantation of a guide cannula. In addition, anchor screws were buried in two positions around the drilled hole. A stainless-steel guide cannula, AG-12 (0.4 mm inside dia., 0.5 mm out. dia., EICOM), was inserted in such a manner that its leading end would be situated in the upper part of the area postrema. For this purpose, the guide cannula was instered from a forward direction at an angle of 20° with the perpendicular (Fig. 46; Note, however, that the drawing shows a microinjection cannula 1.0 mm longer than the guide cannula). With reference to the atlas of Paxinos and Watson (1986), the stereotaxic coordinates were AP: -6.0 mm (from interaural line), L: 0.0 mm, and H: +1.5 mm (from interaural line). The guide cannula was secured to the skull using an instant adhesive, a dental cement, and anchor pieces. A stainless-steel dummy cannula, AD-12 (0.35 mm out. dia., EICOM), was inserted into the guide cannula and locked in position with a cap nut (EICOM). Thereafter, the rats were kept in individual cages.

About a week of feeding after implantation of the guide cannula for postoperative recuperation, an operation was performed for measurements of blood

pressure in conscious state. The rat described above was anesthetized with pentobarbital 50 mg/kg i.p. and immobilized in spine position on a necropsy pad and the left femoral artery was exposed. Polyethylene tubing, SP35 (0.5 mm in. dia., 0.9 mm out. dia., Natsume Seisakusho), was cut to about 60 cm in length and filled with 200 U/ml heparin-containing saline. This tube was inserted about 2.5 cm deep into the femoral artery and secured in position. The free end of the tube was passed under the dorsal skin and exposed in the cervical region (dorsal side).

After waiting overnight postoperatively, the polyethylene tube was connected to a transducer (Spectramed) and the blood pressure was measured. After blood pressure readings became steady, the cap nut and dummy cannula were removed from the rat skull and, instead, a stainless steel microinjection cannula (0.17 mm in. dia., 0.35 mm out. dia., EICOM) connected to a Teflon tube (50 cm long, 0.1 mm in. dia., 0.4 mm out. dia., EICOM) was inserted. The length of the microinjection cannula was adjusted beforehand so that its tip would extend 1 mm from the guide cannula (Fig. 46). One end of the Teflon tube was connected to a microsyringe pump and either PBS or 19P2-L31 dissolved in PBS was injected, in a total volume of 2 µl, into the area postrema at a flow rate of 1.0 µl/min.

After measurement of blood pressure, the microinjection cannula used for injection of 19P2-L31 was disconnected and replaced with a microinjection cannula for injection of a stain (Evans Blue) solution. The stain was infused at the same rate of 1.0 µl/min as the injection of 19P2-L31 for 2 minutes. After a standby time of about 3 minutes, the microinjection cannula was disconnected. The rat was decapitated and the brain was quickly removed and frozen. The brains were cut serial frontal sections on cryostat and the position of

dye infusion was confirmed.

Results of the above experiment showed that injection of 10 nmol of 19P2-L31 into the area postrema of medula oblongata caused an elevation of blood pressure. Typical examples of direct and mean blood pressure are shown in Fig. 47.

[Example 41]

Effects of ligand polypeptide on plasma pituitary hormone level

The inventors explored the effect of 19P2-L31 administered into the third ventricle on pituitary hormone levels in the plasma. Mature male Wistar rats (body weights at operation: 290-350 g) were anesthetized with pentobarbital, 50 mg/kg i.p., and each immobilized in a rat brain stereotaxic apparatus. The incisor bar was set 3.3 mm lower from the interaural line. The skull was exposed, and using a dental drill a hole was made on the bone for implantation of a guide cannula. In addition, an anchor screw was buried in one position around the hole. A stainless-steel guide cannula, AG-12 (0.4 mm in. dia., 0.5 mm out. dia., EICOM), was inserted in such a manner that its tip would be situated in the upper part of the third ventricle. With reference to the atlas of Paxinos and Watson (1986), the stereotaxic coordinates were AP: +7.2 mm (from interaural line), L: 0.0 mm, and H: +2.0 mm (from interaural line). The guide cannula was secured to the skull using an instant adhesive, a dental cement, and an anchor piece. A stainless-steel dummy cannula, AD-12 (0.35 mm out. dia., EICOM), was then passed through the guide cannula and locked in position with a cap nut (EICOM). After the operation the rats were housed in individual cages and kept for at least 3 days for recuperation before starting the experiment.

The operated rat was anesthetized with

pentobarbital 50 mg/kg i.p. and immobilized in dorsal position. After the bilateral jugular veins were exposed, 400 µl of blood was drawn using a 1 ml tuberculin syringe and a 24-G needle (both by Termo). To prevent clotting, the syringe was filled with 20 µl of saline containing 200 U/ml of heparin beforehand. The cap nut and dummy cannula were removed from the rat skull and, instead, a stainless steel microinjection cannula (0.17 mm in. dia., 0.35 mm out. dia., EICOM) connected to Teflon tube (50 cm long, 0.1 mm in. dia., 0.4 mm out. dia., EICOM) was inserted. The length of the microinjection cannula was adjusted beforehand so that its tip would be emergent from the guide cannula by 1 mm. One end of the Teflon tube was connected to a microsyringe pump and either PBS or 19P2-L31 dissolved in PBS was injected, in a total volume of 10 µl, into the third ventricle at a flow rate of 2.5 µl/min. After a standby time of 1 minute following infusion, the microinjection cannula was disconnected and the dummy cannula was reinstated and locked in position with a cap nut. Immediately before initiation of intraventricular administration and 10, 20, 30, 40, and 60 minutes after initiation of administration, 400 µl portions of blood were drawn from the jugular vein. Each blood sample was centrifuged (5,000 rpm, 10 min.) with a high-speed refrigerated microcentrifuge (MR-150, Tommy Seiko) and the supernatant (plasma) was recovered. The amounts of pituitary hormones [prolactin, luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), and growth hormone (GH)] in the plasma were respectively determined by radioimmunoassays.

The results were expressed as a mean±S.E.M. To test for significant difference between the group treated with 19P2-L31 dissolved in PBS and the control group treated with PBS alone, Student's t-test was

used. According to the two-tailed test, $p<0.05$ was assumed to be the minimal level of significance. As shown in Fig. 48, the plasma GH level was significantly decreased at 20 minutes after administration of 50 nmol of 19P2-L31 into the third ventricle, as compared with the control group. Tendencies toward decrease were found at 10, 30, and 40 minutes after administration as well but the changes were not statistically significant. At 60 minutes after administration, there was no difference from the control group. As to plasma prolactin, LH, ACTH, and TSH, none showed significant changes.

[Example 42]

Effects of ligand polypeptide on plasma growth hormone (GH) level in freely moving rats

Mature male Wistar rats were anesthetized with pentobarbital 50 mg/kg i.p. and, as in Example 41, a stainless-steel guide cannula AG-12 (0.4 mm in. dia., 0.5 mm out. dia., EICOM) was implanted in position with its tip situated in the upper part of the third ventricle. After the operation the rats were housed in individual cages and kept for at least 3 days for recuperation and, then, a cannula (30 cm long, 0.5 mm in. dia., 0.9 mm out. dia., Natsume Seisakusho) filled with heparin (200 U/ml)-containing saline was inserted into the right atrium from the right jugular vein under pentobarbital anesthesia. The rats were maintained overnight for complete arousal from anesthesia and then transferred to transparent acrylic cages (30 cm x 30 cm x 35 cm). A 1 ml tuberculin syringe with a 24-G needle (both by Termo) was connected to the cannula inserted in the atrium and 300 µl of blood was drawn. To prevent clotting, the syringe was filled with 20 µl of saline containing 200 U/ml of heparin beforehand. A stainless-steel microinjection cannula (0.17 mm in. dia., 0.35 mm out. dia., EICOM) connected to Teflon

tube (50 cm long, 0.1 mm in. dia., 0.4 mm out. dia., EICOM) was inserted into the guide cannula positioned in the third ventricle. The length of the microinjection cannula was adjusted beforehand so that its tip would be extend 1 mm from the guide cannula. One end of the Teflon tube was connected to a microsyringe pump and either PBS or 19P2-L31 dissolved in PBS was injected, in a total volume of 10 µl, into the third ventricle at a flow rate of 2.5 µl/min. Ten minutes after initiation of administration into the third ventricle, 5 µg/kg GHRH-saline was administered via the cannula inserted into the atrium. Immediately before initiation of intraventricular administration and 10, 20, 30, 40, and 60 minutes after administration of GHRH, 300 µl portions of blood were drawn from the jugular vein. Each blood sample was centrifuged (5,000 rpm, 10 min.) and the supernatant (plasma) was recovered. The concentrations of GH in the plasma were determined by radioimmunoassay.

The results were expressed as a mean±S.E.M. To test for significant difference between the group treated with 19P2-L31 dissolved in PBS and the control group treated with PBS alone, Student's t-test was used. According to the two tailed test, $p<0.05$ was assumed to be the minimal level of significance. As shown in Fig. 49, administration of 5 µg/kg of GHRH elevated the plasma GH level. However, when 50 nmol of 19P2-L31 was administered into the third ventricle, the GHRH-induced elevation of plasma GH was significantly inhibited.

[Example 43]

Preparation of rabbit anti-bovine 19P2-L31 antibodies

Synthetic peptides containing partial 19P2-L31 sequence [peptide-I: SRAHQHSMEIRTPDC (SEQ ID NO:92), peptide-II: CAWYAGRGIRPVGRFNH$_2$ (SEQ ID NO:93), and

peptide-III: CEIRTPDINPAWYAG (SEQ ID NO:94) were conjugated with KLH according to the standard method. Each peptide conjugate (600 μg as a peptide) dissolved in saline was mixed with Freund's complete adjuvant, and the resultant emulsion was subcutaneously injected into three rabbits (NZW, male, 2.5 kg) respectively. Hyperimmunization was carried out three times in total at the same dose of the conjugate as the first injection with Freund's imcomplete adjuvant every three weeks. Antibody titers were determined as follows. Two weeks after the last immunization, blood samples were obtained from the vein of the immunized rabbits respectively. After being incubated at 37°C for 1 hour, the blood samples were kept at 4°C over night. Sera were then prepared by means of centrifugation. An aliquot (100 μl) of each serum sample diluted properly was introduced into 96-well polystyrene microplates which were pre-coated with goat anti-rabbit IgG (Fc) antibodies, and then the microplates were incubated at 4°C for 16 hours. After removing the sera, horse radish peroxidase (HRP)-conjugated peptide-I, II, and III were added to the wells respectively, and then the microplates were incubated at room temperature for 4 hours. After removing the peptides, coloring reaction was done by adding a substrate. The reaction was stopped by adding 100 μl of a stopping solution, and then the absorbance at 450 nm in each well was measured. As shown in Fig. 50, serum samples obtained from the rabbits after the immunization showed binding activities to HRP-conjugated peptides respectively. However, none of binding activities was detected in sera prepared before the immunization. These results indicated that the rabbits received the immunization produced antibodies against peptide-I, II, and III, respectively. To prepare purified IgG antibody fractions, sera obtained from the immunized rabbits was

percipitated with anmonium sulfate. The resultant precipitates were dissolved in borate buffer, and then dialyzed with the same buffer. The IgG fractions thus obtained were then subjected onto affinity columns conjugated with peptide-I or 19P2-L31 respectively. After washing the columns with borate buffer and following with acetate buffer (100 mM, pH 4.5), antibodies bound to the column were eluted with glycine buffer (200 mM, pH 2.0). After being neutralized with 1M Tris, the eluents were used as purified antibodies respectively.

[Example 44]

Inhibitory activity of antibodies against the release of arachidonic acid metabolites induced by 19P2-L31

The purified antibodies prepared as described in Example 43 were tested their inhibitory activity against the release of arachidonic acid metabolites induced by 19P2-L31. The antibodies diluted as indicated in Fig. 51 were mixed with 19P2-L31 (5×10^{-10}M) at room temperature for 1 hour, and then the release of arachidonic acid metabolites was examined as described in Example 11. As shown in Fig. 51, the highest inhibitory activity was observed in anti-peptide-II antibodies.

[Preparation Example 1]

Fifty milligrams of the compound as obtained in Example 21 is dissolved in 50 ml of Japanese pharmacopoeial, distilled water for injection, and Japanese pharmacopoeial, distilled water for injection is added thereto to make 100 ml. The resulting solution is filtered under a germ-free condition, and the filtrate of 1 ml each is filled in vials for injection, freeze-dried and sealed therein also under a germ-free condition.

[Preparation Example 2]

One hundred milligrams of the compound as obtained in Example 21 is dissolved in 50 ml of Japanese pharmacopoeial, distilled water for injection, and Japanese pharmacopoeial, distilled water for injection is added thereto to make 100 ml. The resulting solution is filtered under a germ-free condition, and the filtrate of 1 ml each is filled in vials for injection, freeze-dried and sealed therein also under a germ-free condition.

[Evaluation of the physiological activities of ligand polypeptide of the present invention]

The above examples 37-41 demonstrate that topical administration of ligand polypeptide induces enhancement of spontaneous motor activity and rearing behavior, elevation of body temperature and blood pressure, and decrease in plasma growth hormone concentration. These findings relating to physiological activities are the first proof of various prominent physiologic changes which occur when ligand polypeptide acts on the central nervous system.

Since ligand polypeptide of the present invention, inclusive of its salt, acts on the central nervous systems of warm-blooded animals (e.g. rat, mouse, guinea pig, chicken, rabbit, dog, swine, bovine, sheep, monkey, and man) to induce a variety of pharmacological changes, it is showed that the ligand and salt have the property to alter the intracranial nervous system and endocrine system.

When 19P2-L31 was administered into the lateral ventricle of mice, an increase in the amount of activity was found at the level of 0.01-10 nmol. This fact shows that ligand polypeptide triggers changes in the motor system via the G protein-coupled receptors of the central nervous system. It was also found that administration of the peptide into the lateral

ventricle of mice results in elevation of body temperature and that administration into the area postrema of medula oblongata of rats results in elevation of blood pressure. These actions resemble the pharmacologic actions of known central stimulants (e.g. amphetamine, cocaine, methylphenidate, etc.). Therefore, it is showed that ligand polypeptide or a salt thereof releases biologic amines (dopamine, noradrenaline, serotonin) from the nerve ending reservoirs, in the main (Michio Yuzuru and Takeo Yoshikawa, Medical Science, 42, 535-536, 1991).

Furthermore, when 19P2-L31 was injected into the third ventricle of rats, the plasma growth hormone level was depressed. This finding shows that this peptide acts on the hypothalamus and is associated with secretion of pituitary hormones via the hypothalamo-pituitary system. It is also possible that this peptide directly act on the pituitary so as to suppress the release of growth hormone. Growth hormone releasing hormone (GHRH) which regulates secretion of growth hormone from the hypophysis as well as somatostatin exists in the neighborhood of the third ventricle (Masahiro Tohyama et al., Kagakuteki Shinkeikino Kaibogaku (Chemical Neuroanatomy), 167-216, 1987). Therefore, it is showed that 19P2-L31 is modulating release of these substances.

The above facts show that ligand polypeptide is a peptide acting on the central nervous system to control the autonomous nervous system. The fact that the mRNA of this peptide and of its receptor is expressed at high levels in the hypothalamus and medula oblongata also shows the involvement of ligand polypeptide in the modulation of the autonomous nervous system. In fact, the superior center of autonomous nerve peripherals is the medula oblongata and hypothalamus, where as already elucidated the sympathetic nervous system and the para-

sympathetic nervous system are integrated to play an important role in both neural regulation and humoral regulation.

The above findings indicate the usefulness of ligand polypeptide or an agonist of ligand polypeptide, or a salt thereof, as a central nervous system stimulant causing enhancement of spontaneous motor activity. Thus, the peptide can be used as a prophylactic and/or therapeutic drug for a variety of diseases such as senile dementia, cerebrovascular dementia (dementia due to cerebrovascular disorder), dementia associated with phylodegenerative retroplastic diseases (e.g. Alzheimer's disease, Parkinson's disease, Pick's disease, Huntington's disease, etc.), dementia due to infectious diseases (e.g. delayed viral infections such as Creutzfelt-Jakob disease), dementia associated with endocrine, metabolic, and toxic diseases (e.g. hypothyroidism, vitamin B12 deficiency, alcoholism, and poisoning due to various drugs, metals, or organic compounds), dementia associated with oncogenous diseases (e.g. brain tumor), dementia due to traumatic diseases (e.g. chronic subdural hematoma), depression (melancholia), hyperkinetic (microencephalopathy) syndrome, or disturbance of consciousness. On the other hand, an antagonist of 19P2 ligand or a salt thereof is of value as a CNS deppressant, for instance, and can be used as an antipsychotic drug, an anti-Huntigton's disease drug, an antianxiety drug, or a hypnotic-sedative.

It was made clear that injection of ligand polypeptide into the area postrema of medula oblongata elevates the blood pressure. Therefore, ligand polypeptide or an agonist of ligand polypeptide , or a salt thereof, is of value as a vasopressor. On the other hand, a ligand polypeptide antagonist or a salt thereof is of value as a depressor.

It was found that when ligand polypeptide acts on the hypothalamus, the plasma growth hormone level is depressed. Hypersecretion of growth hormone triggers somatomegaly and acromegalic gigantism (Katamasu et al., Endocrine Syndrome, 78-80, 1993; Hiroi et al., Endocrine Syndrome, 149-151, 1993). Therefore, ligand polypeptide or a ligand polypeptide antagonist, or a salt thereof, can be used as a prophylactic and/or therapeutic drug for somatomegaly and acromegalic gigantism. Moreover, growth hormone promotes release of glucose from the liver and inhibits the uptake of glucose by muscles and adipose tissues from the blood, causing hyperglycemia and diabetes [Eiji Kobayashi, Naibumpi Gensho (Endocrine Phenomena), 1980]. In fact, the secretion of growth hormone is elevated in diabetic patients (Hiroshi Kiyono, Endocrinology and Metabolic Diseases, 385-402, 1994). Therefore, ligand polypeptide or an agonist of ligand polypeptide , or a salt thereof, can be used as a prophylactic and/or therapeutic drug for diabetes, for instance.

On the other hand, an antagonist of ligand polypeptide promotes secretion of growth hormone. Therefore, a ligand polypeptide antagonist or a salt thereof can be used as a prophylactic and/or therapeutic drug for pituitarism leading to a depressed growth hormone level, pituitary dwarfism, and hypoglycemia. Moreover, growth hormone and insulin-like growth factor secreted by growth hormone are effective in amyotrophic lateral sclerosis, osteoporosis, renal failure, and improvement in posto-perative nutritional status (Shizume et al., Endocrine Syndrome, 84-87, 1993, Nikkei Bio-Annal 96, 453-454, 1996; Tobiume et al., Clinical Endocrinology, 44, 1205-1214, 1996). Therefore, a ligand polypeptide antagonist or its salt can be used as a prophylactic and/or therapeutic drug for such illnesses.

[Sequence Listing]

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Takeda Chemical Industries, Ltd.

(B) STREET: 1-1, Doshomachi 4-chome, Chuo-ku

(C) CITY: Osaka

(D) STATE: Osaka

(E) COUNTRY: Japan

(F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: Polypeptides, Their Production and Use

(iii) NUMBER OF SEQUENCES: 94

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:

(B) COMPUTER:

(C) OPERATING SYSTEM:

(D) SOFTWARE:

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Lys Ala Val Gly Ala Trp Leu Leu Cys Leu Leu Leu Leu Gly Leu
 1               5                  10                  15
Ala Leu Gln Gly Ala Ala Ser Arg Ala His Gln His Ser Met Glu Ile
            20                  25                  30
Arg Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg
        35                  40                  45
Pro Val Gly Arg Phe Gly Arg Arg Arg Ala Ala Pro Gly Asp Gly Pro
    50                  55                  60
```

Arg Pro Gly Pro Arg Arg Val Pro Ala Cys Phe Arg Leu Glu Gly Gly
65 70 75 80
Ala Glu Pro Ser Arg Ala Leu Pro Gly Arg Leu Thr Ala Gln Leu Val
85 90 95
Gln Glu

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 294

(B) TYPE: Nucleic acid

(C) STRANDENESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) FEATURE

(C) IDENTIFICATION METHOD: S

(x) SEQUENCE DESCROPTION; SEQ ID NO:2:

ATGAAGGCGG TGGGGGCCTG GCTCCTCTGC CTGCTGCTGC TGGGCCTGGC CCTGCAGGGG 60
GCTGCCAGCA GAGCCCACCA GCACTCCATG GAGATCCGCA CCCCCGACAT CAACCCTGCC 120
TGGTACGCRG GCCGTGGGAT CCGGCCCGTG GGCCGCTTCG GCCGGCGAAG AGCTGCCCYG 180
GGGGACGGAC CCAGGCCTGG CCCCCGGCGT GTGCCGGCCT GCTTCCGCCT GGAAGGCGGY 240
GCTGAGCCCT CCCGAGCCCT CCCGGGGCGG CTGACGGCCC AGCTGGTCCA GGAA 294

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Arg Ala His Gln His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15
Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro Val Gly
20 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg
19

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Arg Ala His Gln His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro Val Gly Arg Phe
20 25 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Arg Ala His Gln His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Arg Ala His Gln His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15
Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30
Arg
33

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro
1 5 10 15
Val Gly Arg Phe
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro
1 5 10 15
Val Gly Arg Phe Gly
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro
1 5 10 15
Val Gly Arg Phe Gly Arg
20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 87

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCAGAGCCC ACCAGCACTC CATGGAGATC CGCACCCCCG ACATCAACCC TGCCTGGTAC 60
GCRGGCCGTG GGATCCGGCC CGTGGGC 87

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 57

(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACCCCCGACA TCAACCCTGC CTGGTACGCR GGCCGTGGGA TCCGGCCCGT GGGCCGC 57

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCAGAGCCC ACCAGCACTC CATGGAGATC CGCACCCCCG ACATCAACCC TGCCTGGTAC 60
GCRGGCCGTG GGATCCGGCC CGTGGGCCGC TTC 93

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGCAGAGCCC ACCAGCACTC CATGGAGATC CGCACCCCCG ACATCAACCC TGCCTGGTAC 60
GCRGGCCGTG GGATCCGGCC CGTGGGCCGC TTCGGC 96

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99
(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGCAGAGCCC ACCAGCACTC CATGGAGATC CGCACCCCCG ACATCAACCC TGCCTGGTAC 60
GCRGGCCGTG GGATCCGGCC CGTGGGCCGC TTCGGCCGG 99

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ACCCCCGACA TCAACCCTGC CTGGTACGCR GGCCGTGGGA TCCGGCCCGT GGGCCGCTTC 60

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACCCCCGACA TCAACCCTGC CTGGTACGCR GGCCGTGGGA TCCGGCCCGT GGGCCGCTTC 60
GGC 63

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACCCCCGACA TCAACCCTGC CTGGTACGCR GGCCGTGGGA TCCGGCCCGT GGGCCGCTTC 60
GGCCGG 66

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 91

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn
1 5 10 15

Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
20 25 30

Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val
35 40 45

Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr
50 55 60

Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr
65 70 75 80

Val Val Leu Val His Pro Leu Arg Arg Arg Ile
85 90

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val Ile Leu Leu
1 5 10 15
Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val Val Pro Gly
20 25 30
Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg Arg Arg Arg
35 40 45
Thr Phe Cys Leu Leu Val Val Val Val Val Val
50 55

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 370

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Ala Ser Ser Thr Thr Arg Gly Pro Arg Val Ser Asp Leu Phe Ser
1 5 10 15
Gly Leu Pro Pro Ala Val Thr Thr Pro Ala Asn Gln Ser Ala Glu Ala
20 25 30
Ser Ala Gly Asn Gly Ser Val Ala Gly Ala Asp Ala Pro Ala Val Thr
35 40 45
Pro Phe Gln Ser Leu Gln Leu Val His Gln Leu Lys Gly Leu Ile Val
50 55 60
Leu Leu Tyr Ser Val Val Val Val Val Gly Leu Val Gly Asn Cys Leu
65 70 75 80
Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn
85 90 95
Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
100 105 110
Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val
115 120 125
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr
130 135 140
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr

```
145                 150                 155                 160
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser
                165                 170                 175
Ala Tyr Ala Val Leu Ala Ile Trp Ala Leu Ser Ala Val Leu Ala Leu
            180                 185                 190
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val
        195                 200                 205
Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu
    210                 215                 220
Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val
225                 230                 235                 240
Ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val
                245                 250                 255
Val Pro Gly Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg
            260                 265                 270
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val Phe Ala
        275                 280                 285
Val Cys Trp Leu Pro Leu His Val Phe Asn Leu Leu Arg Asp Leu Asp
    290                 295                 300
Pro His Ala Ile Asp Pro Tyr Ala Phe Gly Leu Val Gln Leu Leu Cys
305                 310                 315                 320
His Trp Leu Ala Met Ser Ser Ala Cys Tyr Asn Pro Phe Ile Tyr Ala
                325                 330                 335
Trp Leu His Asp Ser Phe Arg Glu Glu Leu Arg Lys Leu Leu Val Ala
            340                 345                 350
Trp Pro Arg Lys Ile Ala Pro His Gly Gln Asn Met Thr Val Ser Val
        355                 360                 365
Val Ile
    370
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu Tyr Asn Val Thr Asn
 1               5                  10                  15
Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
            20                  25                  30
Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val
        35                  40                  45
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Ala Val Thr
    50                  55                  60
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr
65                  70                  75                  80
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser
                85                  90                  95
Ala Tyr Ala Val Leu Ala Ile Trp Val Leu Ser Ala Val Leu Ala Leu
            100                 105                 110
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val
        115                 120                 125
Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu
    130                 135                 140
Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val
145                 150                 155                 160
Ile Leu Leu Ser Tyr Ala Arg Val Ser Val Lys Leu Arg Asn Arg Val
                165                 170                 175
Val Pro Gly Arg Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg
            180                 185                 190
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 126

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser
 1               5                  10                  15
Ala Tyr Ala Val Leu Gly Ile Trp Ala Leu Ser Ala Val Leu Ala Leu
            20                  25                  30
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val
        35                  40                  45
Ser Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Ile
    50                  55                  60
Tyr Ala Trp Gly Leu Leu Leu Gly Thr Tyr Leu Leu Pro Leu Leu Ala
65                  70                  75                  80
Ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val
                85                  90                  95
Val Pro Gly Ser Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg
            100                 105                 110
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 273

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAACG TGACGAACTT CCTCATCGGC   60
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT  120
GCCTTCGAGC CACGCGGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG  180
CAGCCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGGTAC  240
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATC                               273
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 177

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
GGCCTGCTGC TGGTCACCTA CCTGCTCCCT CTGCTGGTCA TCCTCCTGTC TTACGTCCGG    60
GTGTCAGTGA AGCTCCGCAA CCGCGTGGTG CCGGGCTGCG TGACCCAGAG CCAGGCCGAC   120
TGGGACCGCG CTCGGCGCCG GCGCACCTTC TGCTTGCTGG TGGTGGTCGT GGTGGTG      177
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1110

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
ATGGCCTCAT CGACCACTCG GGGCCCCAGG GTTTCTGACT TATTTTCTGG GCTGCCGCCG    60
GCGGTCACAA CTCCCGCCAA CCAGAGCGCA GAGGCCTCGG CGGGCAACGG GTCGGTGGCT   120
GGCGCGGACG CTCCAGCCGT CACGCCCTTC CAGAGCCTGC AGCTGGTGCA TCAGCTGAAG   180
GGGCTGATCG TGCTGCTCTA CAGCGTCGTG GTGGTCGTGG GGCTGGTGGG CAACTGCCTG   240
CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAACG TGACGAACTT CCTCATCGGC   300
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT   360
GCCTTCGAGC CACGCGGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG   420
CAGCCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGCTAC   480
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG   540
CTGGCCATCT GGGCGCTGTC CGCGGTGCTG GCGCTGCCCG CCGCCGTGCA CACCTATCAC   600
GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCGAGGAGT TCTGGGGCTC CCAGGAGCGC   660
CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC   720
```

ATCCTCCTGT CTTACGTCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCTGC 780
GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG 840
GTGGTGGTCG TGGTGGTGTT CGCCGTCTGC TGGCTGCCGC TGCACGTCTT CAACCTGCTG 900
CGGGACCTCG ACCCCCACGC CATCGACCCT TACGCCTTTG GGCTGGTGCA GCTGCTCTGC 960
CACTGGCTCG CCATGAGTTC GGCCTGCTAC AACCCCTTCA TCTACGCCTG GCTGCACGAC 1020
AGCTTCCGCG AGGAGCTGCG CAAACTGTTG GTCGCTTGGC CCCGCAAGAT AGCCCCCCAT 1080
GGCCAGAATA TGACCGTCAG CGTGGTCATC 1110

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 618
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGTACAACG TGACGAATTT CCTCATCGGC 60
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT 120
GCCTTCGAGC CACGCGGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG 180
CAGGCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGCTAC 240
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG 300
CTGGCCATCT GGGTGCTGTC CGCGGTGCTG GCGCTGCCCG CCGCCGTGCA CACCTATCAC 360
GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCGAGGAGT TCTGGGGCTC CCAGGAGCGC 420
CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC 480
ATCCTCCTGT CTTACGCCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCGGGCCGC 540
GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCGCC GGCGCACCTT CTGCTTGCTG 600
GTGGTGGTCG TGGTGGTG 618

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 378
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTGGTTCTGG TGCACCCGCT ACGTCGGCGC ATTTCACTGA GGCTCAGCGC CTACGCGGTG 60
CTGGGCATCT GGGCTCTATC TGCAGTGCTG GCGCTGCCGG CCGCGGTGCA CACCTACCAT 120
GTGGAGCTCA AGCCCCACGA CGTGAGCCTC TGCGAGGAGT TCTGGGGCTC GCAGGAGCGC 180
CAACGCCAGA TCTACGCCTG GGGGCTGCTT CTGGGCACCT ATTTGCTCCC CCTGCTGGCC 240
ATCCTCCTGT CTTACGTACG GGTGTCAGTG AAGCTGAGGA ACCGCGTGGT GCCTGGCAGC 300
GTGACCCAGA GTCAAGCTGA CTGGGACCGA GCGCGTCGCC GCCGCACTTT CTGTCTGCTG 360
GTGGTGGTGG TGGTAGTG 378

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGTGGSCMTS STGGGCAACN YCCTG 25

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTNGWRRGGC ANCCAGCAGA KGGCAAA 27

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTGTGYGYSA TYGCNNTKGA YMGSTAC 27

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AKGWAGWAGG GCAGCCAGCA GANSRYGAA 29

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CTGACTTATT TTCTGGGCTG CCGC 24

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AACACCGACA CATAGACGGT GACC 24

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCICAYCARC AYTGYATGGA 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCIACGGGIC KDATGCCICK GCCIGC 26

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ACGGGCCKDA TGCCICKGCC IGCRTA 26

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CCGGCGTACC AGGCAGGGTT 20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGGCAGGGTT GATGTCGGGG GTGCGGAT 28

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CTGCCAGCAG AGCCCACCAG CACTCCA 27

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GTGGGGGCCT GGCTCCTCTG CCTGCTG 27

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GTGTCGACGA ATGAAGGCGG TGGGGGCCTG GC 32

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGGCTCCCGC TGTTATTCCT GGAC 24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Met Lys Ala Val Gly Ala Trp Leu Leu Cys Leu Leu Leu Leu Gly Leu
1 5 10 15

Ala Leu Gln Gly Ala Ala Ser Arg Ala His Gln His Ser Met Glu Ile
20 25 30

Arg Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly Arg Gly Ile Arg
35 40 45

Pro Val Gly Arg Phe Gly Arg Arg Arg Ala Ala Leu Gly Asp Gly Pro
50 55 60

Arg Pro Gly Pro Arg Arg Val Pro Ala Cys Phe Arg Leu Glu Gly Gly
65 70 75 80

Ala Glu Pro Ser Arg Ala Leu Pro Gly Arg Leu Thr Ala Gln Leu Val
85 90 95

Gln Glu

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 83

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met Ala Leu Lys Thr Trp Leu Leu Cys Leu Leu Leu Leu Ser Leu Val
1 5 10 15

Leu Pro Gly Ala Ser Ser Arg Ala His Gln His Ser Met Glu Thr Arg
20 25 30

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro
35 40 45

Val Gly Arg Phe Gly Arg Arg Arg Ala Thr Pro Arg Asp Val Thr Gly
50 55 60

Leu Gly Gln Leu Ser Cys Leu Pro Leu Asp Gly Arg Thr Lys Phe Ser
65 70 75 80

Gln Arg Gly

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 249

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATGGCCCTGA AGACGTGGCT TCTGTGCTTG CTGCTGCTAA GCTTGGTCCT CCCAGGGGCT 60

TCCAGCCGAG CCCACCAGCA CTCCATGGAG ACAAGAACCC CTGATATCAA TCCTGCCTGG 120

TACACGGGCC GCGGGATCAG GCCTGTGGGC CGCTTCGGCA GGAGAAGGGC AACCCCGAGG 180

GATGTCACTG GACTTGGCCA ACTCAGCTGC CTCCCACTGG ATGGACGCAC CAAGTTCTCT 240

CAGCGTGGA 249

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ser Arg Ala His Gln His Ser Met Glu Thr Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro Val Gly Arg Phe
20 25 30

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Arg Ala His Gln His Ser Met Glu Thr Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ser Arg Ala His Gln His Ser Met Glu Thr Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30

Arg

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe
20

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe Gly
20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Thr Gly Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe Gly Arg
20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

AGCCGAGCCC ACCAGCACTC CATGGAGACA AGAACCCCTG ATATCAATCC TGCCTGGTAC 60
ACGGGCCGCG GGATCAGGCC TGTGGGCCGC TTC 93

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

AGCCGAGCCC ACCAGCACTC CATGGAGACA AGAACCCCTG ATATCAATCC TGCCTGGTAC 60
ACGGGCCGCG GGATCAGGCC TGTGGGCCGC TTCGGC 96

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 99
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
AGCCGAGCCC ACCAGCACTC CATGGAGACA AGAACCCCTG ATATCAATCC TGCCTGGTAC 60
ACGGGCCGCG GGATCAGGCC TGTGGGCCGC TTCGGCAGG                        99
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 60
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```
ACCCCTGATA TCAATCCTGC CTGGTACACG GGCCGCGGGA TCAGGCCTGT GGGCCGCTTC 60
```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 63
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACCCCTGATA TCAATCCTGC CTGGTACACG GGCCGCGGGA TCAGGCCTGT GGGCCGCTTC 60
GGC 63

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ACCCCTGATA TCAATCCTGC CTGGTACACG GGCCGCGGGA TCAGGCCTGT GGGCCGCTTC 60
GGCAGG 66

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 87
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Met Lys Val Leu Arg Ala Trp Leu Leu Cys Leu Leu Met Leu Gly Leu
1 5 10 15
Ala Leu Arg Gly Ala Ala Ser Arg Thr His Arg His Ser Met Glu Ile
20 25 30
Arg Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg
35 40 45
Pro Val Gly Arg Phe Gly Arg Arg Arg Ala Thr Leu Gly Asp Val Pro
50 55 60
Lys Pro Gly Leu Arg Pro Arg Leu Thr Cys Phe Pro Leu Glu Gly Gly
65 70 75 80
Ala Met Ser Ser Gln Asp Gly

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 261

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

ATGAAGGTGC TGAGGGCCTG GCTCCTGTGC CTGCTGATGC TGGGCCTGGC CCTGCGGGGA 60

GCTGCAAGTC GTACCCATCG GCACTCCATG GAGATCCGCA CCCCTGACAT CAATCCTGCC 120

TGGTACGCCA GTCGCGGGAT CAGGCCTGTG GGCCGCTTCG GTCGGAGGAG GGCAACCCTG 180

GGGGACGTCC CCAAGCCTGG CCTGCGACCC CGGCTGACCT GCTTCCCCCT GGAAGGCGGT 240

GCTATGTCGT CCCAGGATGG C 261

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Ser Arg Thr His Arg His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro Val Gly Arg Phe
20 25 30

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Ser Arg Thr His Arg His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Ser Arg Thr His Arg His Ser Met Glu Ile Arg Thr Pro Asp Ile Asn
1 5 10 15

Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro Val Gly Arg Phe Gly
20 25 30

Arg

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe
20

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe Gly
20

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Ser Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe Gly Arg
20

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

AGTCGTACCC ATCGGCACTC CATGGAGATC CGCACCCCTG ACATCAATCC TGCCTGGTAC 60
GCCAGTCGCG GGATCAGGCC TGTGGGCCGC TTC 93

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

AGTCGTACCC ATCGGCACTC CATGGAGATC CGCACCCCTG ACATCAATCC TGCCTGGTAC 60
GCCAGTCGCG GGATCAGGCC TGTGGGCCGC TTCGGT 96

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

AGTCGTACCC ATCGGCACTC CATGGAGATC CGCACCCCTG ACATCAATCC TGCCTGGTAC 60
GCCAGTCGCG GGATCAGGCC TGTGGGCCGC TTCGGTCGG 99

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

ACCCCTGACA TCAATCCTGC CTGGTACGCC AGTCGCGGGA TCAGGCCTGT GGGCCGCTTC 60

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

ACCCCTGACA TCAATCCTGC CTGGTACGCC AGTCGCGGGA TCAGGCCTGT GGGCCGCTTC 60

GGT 63

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

ACCCCTGACA TCAATCCTGC CTGGTACGCC AGTCGCGGGA TCAGGCCTGT GGGCCGCTTC 60

GGTCGG 66

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(ix) FEATURE: Xaa of the 10th position is Ala or Thr. Xaa of the 11th position is Gly or Ser. Xaa of the 21st position is H, Gly or GlyArg.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Thr Pro Asp Ile Asn Pro Ala Trp Tyr Xaa Xaa Arg Gly Ile Arg Pro
1 5 10 15

Val Gly Arg Phe Xaa
20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(ix) FEATURE: Xaa of the 3rd position is Ala or Thr. Xaa of the 5th position is Gln or Arg. Xaa of the 10th position is Ile or Thr.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Ser Arg Xaa His Xaa His Ser Met Glu Xaa Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CARCAYTCCA TGGAGACAAG AACCCC 26

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

TACCAGGCAG GATTGATACA GGGG 24

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GGCATCATCC AGGAAGACGG AGCAT 25

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

AGCAGAGGAG AGGGAGGGTA GAGGA 25

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

ACGTGGCTTC TGTGCTTGCT GC 22

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GCCTGATCCC GCGGCCCGTG TACCA 25

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TTGCCCTTCT CCTGCCGAAG CGGCCC 26

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GGCGGGGGCT GCAAGTCGTA CCCATCG 27

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CGGCACTCCA TGGAGATCCG CACCCCT 27

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CAGGCAGGAT TGATGTCAGG GGTGCGG 27

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

CATGGAGTGC CGATGGGTAC GACTTGC 27

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GGCCTCCTCG GAGGAGCCAA GGGATGA 27

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GGGAAAGGAG CCCGAAGGAG AGGAGAG 27

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CCTGCTGGCC ATTCTCCTGT CTTAC 25

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GGGTCCAGGT CCCGCAGAAG GTTGA 25

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GAAGACGGAG CATGGCCCTG AAGAC 25

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GGCAGCTGAG TTGGCCAAGT CCAGT 25

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Ser Arg Ala His Gln His Ser Met Glu Ile Arg Thr Pro Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Cys Ala Trp Tyr Ala Gly Arg Gly Ile Arg Pro Val Gly Arg Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: Amino acid

(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Cys Glu Ile Arg Thr Pro Asp Ile Asn Pro Ala Trp Tyr Ala Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GTTCACAGGT CGACATGACC TCAC 24

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

CTCAGAGCTA GCAGAGTGTC ATCAG 25